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FILE 'HOME' ENTERED AT 14:40:22 ON 30 NOV 2005

=> ndex bioscience medicine Dissabs
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=> index bioscience medicine Dissabs
FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                        ENTRY    SESSION
FULL ESTIMATED COST                                      0.21       0.21

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE,
      AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS,
      BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB,
      CROPU, DDFB, DDFU, DGENE, DISSABS, ...' ENTERED AT 14:41:06 ON 30 NOV 2005

77 FILES IN THE FILE LIST IN STNINDEX

Enter SET DETAIL ON to see search term postings or to view
search error messages that display as 0* with SET DETAIL OFF.

=> s (induc? or stimulat? enhance? increas?) (S)(programmed cell death or apoptosis) (P)
(administer? or deliver?)
        26   FILE ADISCTI
       153   FILE ADISINSIGHT
        34*  FILE ADISNEWS
        19   FILE AGRICOLA
         1*  FILE ANTE
         0*  FILE AQUALINE
         5   FILE AQUASCI
         5   FILE BIOBUSINESS
         3*  FILE BIOCOMMERCE
       164*  FILE BIOENG
      1459   FILE BIOSIS
 12 FILES SEARCHED...
      1105*  FILE BIOTECHABS
      1105*  FILE BIOTECHDS
       823*  FILE BIOTECHNO
 15 FILES SEARCHED...
       121   FILE CABA
       798   FILE CANCERLIT
      1942   FILE CAPLUS
         6*  FILE CEABA-VTB
        11*  FILE CIN
         2   FILE CONFSCI
         3   FILE CROPU
       354   FILE DDFU
 26 FILES SEARCHED...
     19001   FILE DGENE
 27 FILES SEARCHED...
       110   FILE DISSABS
       957   FILE DRUGU
        54   FILE EMBAL
      1377   FILE EMBASE
      1588*  FILE ESBIOBASE
 34 FILES SEARCHED...
       313*  FILE FEDRIP
         0*  FILE FOMAD
         0*  FILE FOREGE
         6*  FILE FROSTI
         9*  FILE FSTA
         9   FILE GENBANK
      1185   FILE IFIPAT
        45   FILE IMSDRUGNEWS
        98   FILE IMSRESEARCH
       104   FILE JICST-EPLUS
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          5*  FILE KOSMET
        638   FILE LIFESCI
       1517   FILE MEDLINE
          8   FILE NIOSHTIC
  50 FILES SEARCHED...
         29*  FILE NTIS
          0*  FILE NUTRACEUT
          1   FILE OCEAN
       1097*  FILE PASCAL
  54 FILES SEARCHED...
         41   FILE PHAR
          9*  FILE PHARMAML
         14   FILE PHIN
        114   FILE PROMT
          9   FILE PROUSDDR
       1347   FILE SCISEARCH
          2   FILE SYNTHLINE
       2095   FILE TOXCENTER
       2726   FILE USPATFULL
  67 FILES SEARCHED...
        284   FILE USPAT2
          2   FILE VETU
          1*  FILE WATER
       1292   FILE WPIDS
  72 FILES SEARCHED...
          8   FILE WPIFV
       1292   FILE WPINDEX
         25   FILE IPA
          2   FILE NAPRALERT
         76   FILE NLDB

  60 FILES HAVE ONE OR MORE ANSWERS,   77 FILES SEARCHED IN STNINDEX

L1    QUE (INDUC? OR STIMULAT? ENHANCE? INCREAS?) (S)(PROGRAMMED CELL DEATH OR A
      POPTOSIS) (P) (ADMINISTER? OR DELIVER?)

=> D rank
F1       19001   DGENE
F2        2726   USPATFULL
F3        2095   TOXCENTER
F4        1942   CAPLUS
F5        1588*  ESBIOBASE
F6        1517   MEDLINE
F7        1459   BIOSIS
F8        1377   EMBASE
F9        1347   SCISEARCH
F10       1292   WPIDS
F11       1292   WPINDEX
F12       1185   IFIPAT
F13       1105*  BIOTECHABS
F14       1105*  BIOTECHDS
F15       1097*  PASCAL
F16        957   DRUGU
F17        823*  BIOTECHNO
F18        798   CANCERLIT
F19        638   LIFESCI
F20        354   DDFU
F21        313*  FEDRIP
F22        284   USPAT2
F23        164*  BIOENG
F24        153   ADISINSIGHT
F25        121   CABA
F26        114   PROMT
F27        110   DISSABS
F28        104   JICST-EPLUS
F29         98   IMSRESEARCH
F30         76   NLDB
F31         54   EMBAL
F32         45   IMSDRUGNEWS
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F33          41   PHAR
F34          34*  ADISNEWS
F35          29*  NTIS
F36          26   ADISCTI
F37          25   IPA
F38          19   AGRICOLA
F39          14   PHIN
F40          11*  CIN
F41           9   GENBANK
F42           9   PROUSDDR
F43           9*  FSTA
F44           9*  PHARMAML
F45           8   NIOSHTIC
F46           8   WPIFV
F47           6*  CEABA-VTB
F48           6*  FROSTI
F49           5   AQUASCI
F50           5   BIOBUSINESS
F51           5*  KOSMET
F52           3   CROPU
F53           3*  BIOCOMMERCE
F54           2   CONFSCI
F55           2   SYNTHLINE
F56           2   VETU
F57           2   NAPRALERT
F58           1   OCEAN
F59           1*  ANTE
F60           1*  WATER


=> FIL F3-4 F6-12 F16 F18-20 F22 F24-32
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                               12.98      13.19

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=> s L1 and (administer? (s) (peptide? or polypeptide? or protein? or enzyme?)
UNMATCHED LEFT PARENTHESIS 'AND (ADMINISTER'
The number of right parentheses in a query must be equal to the
number of left parentheses.

=> s L1 and (administer? (s) (peptide? or polypeptide? or protein? or enzyme?) )
L2          63 FILE TOXCENTER
L3          66 FILE CAPLUS
L4          32 FILE MEDLINE
L5          41 FILE BIOSIS
L6          29 FILE EMBASE
L7          35 FILE SCISEARCH
L8         578 FILE WPIDS
L9         349 FILE IFIPAT
L10        161 FILE DRUGU
L11        172 FILE CANCERLIT
L12        110 FILE LIFESCI
L13        181 FILE USPAT2
L14         77 FILE ADISINSIGHT
L15         31 FILE CABA
L16         17 FILE PROMT
L17         15 FILE DISSABS
L18         20 FILE JICST-EPLUS
L19         24 FILE IMSRESEARCH
L20         13 FILE NLDB
L21          9 FILE EMBAL
L22          5 FILE IMSDRUGNEWS

TOTAL FOR ALL FILES
L23       2028 L1 AND (ADMINISTER? (S) (PEPTIDE? OR POLYPEPTIDE? OR PROTEIN?
               OR ENZYME?) )

=> (induc? or stimulat? enhance? increas?) (S)(programmed cell death or apoptosis) (P)
((administer? or deliver?) (A) (peptide? or polypeptide? or protein? or enzyme?) )
(INDUC? IS NOT A RECOGNIZED COMMAND
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"HELP COMMANDS" at an arrow prompt (=>).

=> s (induc? or stimulat? enhance? increas?) (S)(programmed cell death or apoptosis) (P)
((administer? or deliver?) (A) (peptide? or polypeptide? or protein? or enzyme?))
L24 10 FILE TOXCENTER
L25 15 FILE CAPLUS
L26 9 FILE MEDLINE
L27 8 FILE BIOSIS
L28 8 FILE EMBASE
L29 8 FILE SCISEARCH
L30 7 FILE WPIDS
L31 6 FILE IFIPAT
L32 8 FILE DRUGU
L33 0 FILE CANCERLIT
L34 3 FILE LIFESCI
L35 9 FILE USPAT2
L36 0 FILE ADISINSIGHT
L37 0 FILE CABA
L38 0 FILE PROMT
L39 2 FILE DISSABS
L40 1 FILE JICST-EPLUS
L41 0 FILE IMSRESEARCH
L42 6 FILE NLDB
L43 0 FILE EMBAL
L44 0 FILE IMSDRUGNEWS

TOTAL FOR ALL FILES
L45 100 (INDUC? OR STIMULAT? ENHANCE? INCREAS?) (S)(PROGRAMMED CELL
DEATH OR APOPTOSIS) (P) ((ADMINISTER? OR DELIVER?) (A) (PEPTIDE?
OR POLYPEPTIDE? OR PROTEIN? OR ENZYME?))

=> dup rem l45
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ANSWERS FROM THESE FILES WILL BE CONSIDERED UNIQUE
PROCESSING COMPLETED FOR L45
L46 58 DUP REM L45 (42 DUPLICATES REMOVED)

=> d l46 1-58 ibib abs

L46 ANSWER 1 OF 58 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2005:207496 CAPLUS
DOCUMENT NUMBER: 143:301558
TITLE: Carbon nanotubes as intracellular protein transporters: Generality and biological functionality
AUTHOR(S): Kam, Nadine Wong Shi; Dai, Hongjie
CORPORATE SOURCE: Department of Chemistry and Laboratory for Advanced Materials, Stanford University, Stanford, CA, 94305, USA
SOURCE: Los Alamos National Laboratory, Preprint Archive, Condensed Matter (2005) 1-22, arXiv:cond-mat/0503005, 1 Mar 2005
CODEN: LNCMFR
URL: http://xxx.lanl.gov/pdf/cond-mat/0503005
PUBLISHER: Los Alamos National Laboratory
DOCUMENT TYPE: Preprint
LANGUAGE: English
AB Various proteins adsorb spontaneously on the sidewalls of acid-oxidized single-walled carbon nanotubes. This simple non-specific binding scheme can be used to afford non-covalent protein-nanotube conjugates. The proteins are found to be readily transported inside various mammalian cells with nanotubes acting as the transporter via the endocytosis pathway. Once released from the endosomes, the internalized protein-nanotube conjugates can enter into the cytoplasm of cells and perform biol. functions as shown by **apoptosis induction** by transported cytochrome c. Carbon nanotubes represent a new class of mol. transporters potentially useful for future in-vitro and in-vivo **protein delivery** applications.
REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS

L46 ANSWER 2 OF 58 DISSABS COPYRIGHT (C) 2005 ProQuest Information and Learning Company; All Rights Reserved on STN

ACCESSION NUMBER: 2005:60743 DISSABS Order Number: AAI3168441
TITLE: Tackling ErbB2: ErbB2-targeting peptide therapy and combination therapy with trastuzumab plus PI3K inhibitors
AUTHOR: Lan, Keng-Hsueh [Ph.D.]; Yu, Dihua [advisor]
CORPORATE SOURCE: The University of Texas Health Science Center at Houston Graduate School of Biomedical Sciences (2034)
SOURCE: Dissertation Abstracts International, (2005) Vol. 66, No. 3B, p. 1305. Order No.: AAI3168441. 112 pages. ISBN: 0-542-04213-4.
DOCUMENT TYPE: Dissertation
FILE SEGMENT: DAI
LANGUAGE: English
ENTRY DATE: Entered STN: 20051123
Last Updated on STN: 20051123

AB ErbB2 is an excellent target for cancer therapies because its overexpression was found in about 30% of breast cancers and correlated with poor prognosis of the patients. Unfortunately, current therapies for ErbB2-positive breast cancers remain unsatisfying due to side effects and resistance, and new therapies for ErbB2 overexpressing breast cancers are needed. Peptide/protein therapy using cell-penetrating peptides (CPPs) as carriers is promising because the internalization is highly efficient and the cargos can be bioactive. The major obstacle in using CPPs for therapy is their lack of specificity. We sought to develop a peptide carrier specifically introducing therapeutics to ErbB2-overexpressing breast cancer cells. By modifying the TAT-derived CPP, and attaching anti-HER2/neu peptide mimetic (AHNP), we developed the peptide carrier (P3-AHNP) specifically targeted ErbB2-overexpressing breast cancers in vitro and in vivo. A STAT3 SH2 domain-binding peptide conjugated to this peptide carrier (P3-AHNP-STAT3BP) was delivered preferentially into ErbB2-overexpressing breast cancer cells in vitro and in vivo. P3-AHNP-STAT3BP inhibited growth and **induced apoptosis** in vitro, with ErbB2-overexpressing 435.eB cells being more sensitive than the ErbB2-lowexpressing MDA-MB-435 cells. P3-AHNP-STAT3BP preferentially accumulated and inhibited growth in 435.eB xenografts, comparing with MDA-MB-435 xenografts or normal tissues with low levels of ErbB2. This ErbB2-targeting **peptide delivery** system provided the basis for future development of novel cancer target-specific treatments with low toxicity to normal cells.

Another urgent issue in treating ErbB2-positive breast cancers is trastuzumab resistance. Trastuzumab is the only FDA-approved ErbB2-targeting antibody for treatment of metastatic breast cancers overexpressing ErbB2, and has remarkable therapeutic efficacy in certain patients. The overall trastuzumab response rate, however, is limited, and understanding the mechanisms of trastuzumab resistance is needed to overcome this problem. We report that PTEN activation contributes to trastuzumab's anti-tumor activity. Trastuzumab treatment quickly inactivated Src, which reduced PTEN tyrosine phosphorylation, increased PTEN membrane localization and its phosphatase activity in cancer cells. Reducing PTEN expression in breast cancer cells by antisense oligonucleotides conferred trastuzumab resistance in vitro and in vivo. Importantly, PI3K inhibitors sensitized PTEN-deficient breast cancers to the growth inhibition by trastuzumab in vitro and in vivo, suggesting that combination therapies with PI3K inhibitors plus trastuzumab could overcome trastuzumab resistance.

L46 ANSWER 3 OF 58 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN

ACCESSION NUMBER: 2005-488034 [49] WPIDS
DOC. NO. CPI: C2005-148715
TITLE: New delivery peptides useful for the production of a composition for the treatment of e.g. Crohn's disease, ulcerative colitis, gastrointestinal ulcers, abnormal proliferative diseases, cystic fibrosis, asthma and allergic rhinitis.
DERWENT CLASS: B04 B05
INVENTOR(S): MEIJER, H; STURM, B; WEHLING, P; REINECKE, J

PATENT ASSIGNEE(S): (ORTH-N) ORTHOGEN AG
COUNTRY COUNT: 108
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2005054279	A1	20050616	(200549)*	EN	89

RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IS IT KE LS LU MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW

DE 10355559	A1	20050623	(200549)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2005054279	A1	WO 2004-EP13203	20041119
DE 10355559	A1	DE 2003-10355559	20031121

PRIORITY APPLN. INFO: DE 2003-10355559 20031121
AN 2005-488034 [49] WPIDS
AB WO2005054279 A UPAB: 20050802
NOVELTY - **Delivery peptide** (A) comprising an amino acid sequence (I), is new.

DETAILED DESCRIPTION - **Delivery peptide** (A) comprising an amino acid sequence (I) of formula ((K)nA1B1C1(K)mA2B2C2(K)lA3B3C3(K)o), is new.

K = lysine (K);

B1-B3 = arginine (R), glutamine (Q) or histidine (H);

A1-A3, C1-C3 = arginine (R), histidine (H) or missing; and

n, m, l, o = 0-5.

The total number of amino acid residues is not more than 10.

INDEPENDENT CLAIMS are also included for:

(1) an expression cassette comprising a DNA transgen encoding a fusion protein comprising a leader sequence, a protein of interest and (A);

(2) a transfer vector comprising the expression cassette;

(3) a peptide-cargo complex (A1) comprising (A) and at least one cargo molecule (II);

(4) a pharmaceutical composition comprising (A1) (comprising a biologically active or therapeutic agent) and a carrier;

(5) a method for the delivery of a cargo to the surface of, into or across a biological barrier, comprising providing a cargo and at least one (A), forming (A1), contacting the barrier with (A1) and delivering the cargo to the surface of, into or across the barrier; and

(6) a method for eliciting an immune response in an animal or a human body comprising administration of an immunogen comprising (A1) to a target cell of the body.

ACTIVITY - Antiinflammatory; Antiulcer; Gastrointestinal-Gen.; Cytostatic; CNS-Gen.; Respiratory-Gen.; Antiasthmatic; Antiallergic; Vasotropic; Antiparkinsonian; Neuroleptic; Anti-HIV; Anticonvulsant; Neuroprotective; Vulnerary; Antidepressant; Nootropic; Antimigraine; Analgesic; Cardiovascular-Gen.; Osteopathic; Muscular-Gen.; Antiarthritic; Dermatological.

MECHANISM OF ACTION - None given.

USE - (A) is useful for the production of a kit for cellular internalisation of a linked cargo molecule or for nuclear translocation in a target cell or for translocation in the mitochondria of a target cell in an animal or a human body. (A) is useful for the production of a composition for the treatment of Crohn's disease, ulcerative colitis, gastrointestinal ulcers, peptic ulcer disease, abnormal proliferative diseases, an infection with Helicobacter pylori, cystic fibrosis, asthma, allergic rhinitis, chronic obstructive pulmonary disease, ischemia,

Parkinson's disease, schizophrenia, cancer, AIDS, infections of the central sclerosis, epilepsy, multiple sclerosis, neurodegenerative disease, trauma, depression, Alzheimer's disease, migraine, pain or seizure disorders. (A) is useful for the production of a composition for the treatment of glucocerebrosidase deficiency (Gaucher's disease), mucopolysaccharidosis I, sanfilippo B syndrome, pancreatic insufficiency, severe combined immunodeficiency syndrome, neuromuscular dysfunction associated with triose phosphate isomerase deficiency, inflammatory, degenerative joint and spine diseases, arthritis, (osteoarthritis), low back pain, bone repair, fracture healing, therapy of muscle or ligament injury. (II) is useful as a therapeutic agent for a condition (Crohn's disease, ulcerative colitis, gastrointestinal ulcers, peptic ulcer disease or abnormal proliferative diseases). (II) (H2-histamine inhibitor, an inhibitor of the proton-potassium ATPase or an antibiotic directed at Helicobacter pylori) is useful as a therapeutic for ulcers. (II) is useful as a therapeutic agent for treating a bronchial condition (cystic fibrosis, asthma, allergic rhinitis or chronic obstructive pulmonary disease), ischemia, Parkinson's disease, schizophrenia, cancer, AIDS, infections of the central nervous system, epilepsy, multiple sclerosis, neurodegenerative disease, trauma, depression, Alzheimer's disease, migraine, pain, seizure disorders, inflammatory, degenerative joint and spine diseases, arthritis, (osteoarthritis), low back pain, bone repair, fracture healing, therapy of muscle or ligament injury). (A1) is useful for the production of a composition to **induce** synovial cell death or **apoptosis** in a tumor cell. (A1) is useful for the production of a composition to reduce white blood cells in arthritic joints or to reduce the effects of skin aging. (A1) is useful for the production of a immunogen for eliciting an immune response in an animal or a human body (all claimed).

ADVANTAGE - (A) enhances transport or delivery and do not affect or hinder the biological activity of the cargo molecule. The biological activity of the molecule associated with (A), forming a peptide-cargo conjugate or complex, is substantially the same as of the isolated cargo in the absence of (A). There is no need for the cargo to become active, to cleave off the **delivery peptide** as soon as the peptide-cargo conjugate or complex has been internalized into the target tissue or cell or has reached or crossed the biological barrier towards its target. (A) readily delivers conjugated biotin into the skin. The ability of (A) to deliver conjugated biotin into the skin was tested. The results showed that the conjugated biotin was transported into and across the epidermis, and into the dermis by all peptides and thus (A) delivered conjugated biotin into skin and no significant difference in translocation was observed.

Dwg.0/8

L46 ANSWER 4 OF 58 DRUGU COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2005-34152 DRUGU P
TITLE: Induction of neutralizing antibodies and Th1-polarized and CD4-independent CD8+ T-cell responses following delivery of human immunodeficiency virus type 1 Tat protein by recombinant adenylate cyclase of Bordetella pertussis.
AUTHOR: Mascarell L; Fayolle C; Bauche C; Ladant D; Leclerc C
CORPORATE SOURCE: INSERM; Inst.Pasteur-Paris
LOCATION: Paris, Fr.
SOURCE: J.Virol. (79, No. 15, 9872-84, 2005) 7 Fig. 2 Tab. 73 Ref.
CODEN: JOVIAM ISSN: 0022-538X
AVAIL. OF DOC.: Biologie des Regulations Immunitaires, INSERM E 352, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris cedex 15, France. (C.L.; e-mail: cleclerc@pasteur.fr).
LANGUAGE: English
DOCUMENT TYPE: Journal
FIELD AVAIL.: AB; LA; CT
FILE SEGMENT: Literature
AN 2005-34152 DRUGU P
AB I.p., s.c. or intradermal immunization of mice with a prototype vaccine based on the HIV type 1 Tat delivered by adenylate cyclase (CyaA) from Bordetella pertussis (CyaA-E5-Tat) in the absence of adjuvant elicited strong and long-lasting neutralizing anti-Tat antibody responses more efficiently than those obtained after immunization with Tat toxoid in

aluminum hydroxide (alum; Serva) adjuvant. CyaA-E5-Tat induced a Th1-polarized immune response in contrast to the Th2-polarized immune responses obtained with the Tat toxoid. HIV-Tat-specific gamma interferon-producing CD8+ T cells were generated after vaccination with CyaA-E5-Tat in a CD4+ T-cell-independent manner. CyaA-E5-Tat represents an attractive vaccine candidate for both preventive and therapeutic vaccination involving CyaA as an efficient nonreplicative vector for **protein delivery**.

ABEX Methods Balb/c mice (aged 6-6 wk) were i.p., s.c. or intradermally immunized with CyaA-E5 or CyaA-E5-Tat. Results A recombinant CyaA carrying the full HIV-1 Tat protein (CyaA-E5-Tat) was designed. P4 cells were incubated for 2 hr with recombinant Tat or CyaA-E5-Tat; Tat strongly increased beta-Gal production by P4 cells due to its transactivating function. CyaA-E5-Tat was unable to **induce** beta-Gal expression. CyA-wt **induced apoptosis** of BM-DCs, whereas CyaA-E5 and CyaA-E5-Tat were not toxic for these cells. Immunization with HIV-1 Tat delivered by CyaA **induced** a strong and long-lasting anti-Tat humoral response. Immunization with CyaA-E5-Tat by the intradermal route optimized the immune response. Mice immunized with either CyaA-E5-Tat or Tat toxoid maintained a high antibody titer, demonstrating the persistence of the humoral immune response. Analyses of the anti-Tat immunoglobulin G isotypes and the cytokine pattern showed that CyaA-E5-Tat **induced** a Th1-polarized immune response in contrast to the Th2-polarized immune responses obtained with the Tat toxoid. Immunization with CyaA-E5-Tat elicited HIV-Tat-specific interferon-gamma producing CD8+ T-cells independently of CD4+ T-cell help. The Tat epitope containing amino acids 46-65 and the Tat toxoid still **induced** IFN-gamma producing cells after depletion of CD8+ T-cells. Immunization with CyaA-E5-Tat triggered CD8+ T-cells responses in mice depleted of CD4+ T-cells by i.p. injection of rat anti-CD4+ mAbs. (ECB/E161)

L46 ANSWER 5 OF 58 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 2005:274260 CAPLUS
DOCUMENT NUMBER: 142:478285
TITLE: Carbon nanotubes as intracellular protein transporters: Generality and biological functionality
AUTHOR(S): Kam, Nadine Wong Shi; Dai, Hongjie
CORPORATE SOURCE: Department of Chemistry and Laboratory for Advanced Materials, Stanford University, Stanford, CA, 94305, USA
SOURCE: Journal of the American Chemical Society (2005), 127(16), 6021-6026
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Various proteins adsorb spontaneously on the sidewalls of acid-oxidized single-walled carbon nanotubes. This simple nonspecific binding scheme can be used to afford noncovalent protein-nanotube conjugates. The proteins are found to be readily transported inside various mammalian cells with nanotubes acting as the transporter via the endocytosis pathway. Once released from the endosomes, the internalized protein-nanotube conjugates can enter into the cytoplasm of cells and perform biol. functions, evidenced by **apoptosis induction** by transported cytochrome c. Carbon nanotubes represent a new class of mol. transporters potentially useful for future in vitro and in vivo **protein delivery** applications.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L46 ANSWER 6 OF 58 DRUGU COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2005-35623 DRUGU P
TITLE: Ubiquitination-resistant p53 protein transduction therapy facilitates anti-cancer effect on the growth of human malignant glioma cells.
AUTHOR: Michiue H; Tomizawa K; Matsushita M; Tamiya T; Lu Y F; Ichikawa T; Date I; Matsui H
LOCATION: Okayama; Kagawa, Jap.

SOURCE: FEBS Lett. (579, No. 18, 3965-69, 2005) 6 Fig. 18 Ref.
CODEN: FEBLAL ISSN: 0014-5793
AVAIL. OF DOC.: Dept. of Physiol., Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8558, Japan. (K.T.). (e-mail: tomikt@md.okayama-u.ac.jp).
LANGUAGE: English
DOCUMENT TYPE: Journal
FIELD AVAIL.: AB; LA; CT
FILE SEGMENT: Literature
AN 2005-35623 DRUGU P
AB Protein transduction therapy (PTT) using poly-arginine can deliver the bioactive p53 protein into cancer cells inhibiting their proliferation. However, a disadvantage of such therapy is the short intracellular half-life of the **delivered protein**. Mutant p53 protein (6KR-p53), generated by substituting multiple lysine residues in the C-terminal with arginine, was effectively delivered to human malignant glioma cells, was resistant to Mdm2-mediated ubiquitination, and showed higher transcription regulatory activity and powerful inhibition of glioma cell proliferation. PTT of ubiquitin-proteasome-mediated, degradation-resistant p53 proteins, such as 6KR-p53, may avoid the disadvantages of p53 PTT for cancer.
ABEX After protein transduction, a high level of WT-p53 was detected in A172, U251-MG and T98G cells but the level was undetectable in U251-MG and T98 cells at 24 hr and in A172 cells 48 hr after the protein transduction. 3KR-p53 and 6KR-p53 were maintained at a high level after 24 hr protein transduction. WT-p53 transduced A172 cells showed migration of p53 was consistent with ubiquitination. WT-p53-transduced U251-MG cells showed slower migration forms of p53 were observed in presence of MG132. 6KR-p53-transduced cells showed slower migrating forms of p53 were not detected in presence and absence of MG132. The transcription regulatory activity of 3KR-p53 and 6KR-p53 was higher than that of WT-p53 and Transduction resulted in maintenance of higher p53 activity after 48 hr vs. Transduction of WT-p53. WT-p53 protein transduction **induced** the expression of endogenous p21 in the U251-MG cells. 3KR-p53 was more effective for **inducing** p21 expression. In the 6KR-p53 transduced cells endogenous p21 expression was most prominent. WT-p53 (1-100 nM) did not inhibit the cell growth of T98G, U251-MG and A172 cells. 3KR-p53 inhibited the proliferation and failed to inhibit cell growth of U251-MG and A172 cell lines. 6KR-p53 (100 nM) inhibited cell growth of T98G, U251-MG and A172 cells after 96 hr protein transduction. 6KR-p53 **induced apoptosis** in cells vs. WT-p53. The transduction of 3KR-p53 and 6KR-p53 did not inhibit the growth of primary astrocytes and **induced apoptosis**. Recombinant adenovirus carrying the wild-type p53 gene **induced apoptosis** and inhibited the cell growth of primary astrocytes. (GA/CW)

L46 ANSWER 7 OF 58 DRUGU COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2005-17936 DRUGU P
TITLE: Tat-neutralizing versus Tat-protecting antibodies in rhesus macaques vaccinated with Tat peptides.
AUTHOR: Belliard G; Hurtrel B; Moreau E; Lafont B A P; Monceaux V; Roques B; Desgranges C; Aubertin A M; Le Grand R; Muller S
CORPORATE SOURCE: CNRS-Inst.Mol.Cell.Biol.Strasbourg; Inst.Pasteur-Paris; INSERM-Inst.Mol.Genet.Paris; INSERM-Inst.Virol.Strasbourg; INSERM
LOCATION: Strasbourg, Paris; Fontenay Aux Roses, Fr.
SOURCE: Vaccine (23, No. 11, 1399-407, 2005) 5 Fig. 35 Ref.
CODEN: VACCDE ISSN: 0264-410X
AVAIL. OF DOC.: CNRS UPR9021, Institut de Biologie Moleculaire et Cellulaire, 15 rue Rene Descartes, 67000 Strasbourg, France. (S.M.). (e-mail: S.Muller@ibmc.u-strasbg.fr).
LANGUAGE: English
DOCUMENT TYPE: Journal
FIELD AVAIL.: AB; LA; CT
FILE SEGMENT: Literature
AN 2005-17936 DRUGU P
AB I.m. and intranasal (i.n.) immunization with a cocktail of 3 Tat peptides

encompassing residues 1-20, 1-61, and 44-61 (PA, PB, and PC, respectively) protected only 1 monkey from intrarectal simian HIV BX08 challenge. The protected animal showed the highest levels of CD3+, CD4+, CD8+, and CD20+ cells. All immunized animals, except 1, gave a good cross-reactive Ab response to Tat but the proliferative response and levels of IL-2 secretion by peripheral blood mononuclear cells (PBMC) recalled ex-vivo with active Tat protein were weak. These findings support the view that actively generated Ab able to interfere with Tat transactivation and Tat-**induced apoptosis** are not sufficient for conferring protection against a viral challenge.

ABEX Methods Male rhesus macaques (Macaca mulatta, 3.5-9.5 kg) were immunized i.m. and i.n. with a cocktail of 3 Tat peptides (PA, PB, and PC, respectively; 50 of each **peptide**) **administered** in Montanide ISA 720 adjuvant (group A). Controls received adjuvant alone. Animals were challenged with intrarectal 10 MID50 of simian HIV BX08 10 wk after the last booster immunization (day 203). Results In group A, all animals, except 1, developed cross-reactive Ab response to cognate protein Tat. These animals also developed elevated IgG Ab titers reacting in ELISA and with PA, PB, and PC. Ab from 4 animals blocked 14%-31% of Tat-dependent long-terminal repeat transactivation, and Ab from 2 other animals inhibited up to 65% and 95% of Tat activity, respectively. Ex-vivo, in the case of 1 animal (animal A), there was marked proliferation of PBMC in the presence of PB and PC. When the Tat protein was used as recall antigen, low levels of IFN-gamma and no proliferation were detectable in the culture of PBMC from animal A. Upon challenge, except for 1 animal (animal B), all group A animals were infected with peaks of infection between days 17-28. Viral RNA remained undetectable in the plasma of animal B for up to 59 days postchallenge. Postchallenge, the level of IgG reacting in ELISA with PA, PB, and PC and with Tat protein dropped. The proliferative response of PBMC of 1 animal (animal A) that was elevated after challenge in the presence of Tat decreased with time. IFN-gamma production dropped in cultures of PBMC from animal A, and remained negative in the case of other animals. Animal A showed the highest absolute number of CD3+, CD4+, CD8+, and CD20+ cells. (ABD/CW)

L46 ANSWER 8 OF 58 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 2

ACCESSION NUMBER: 2005:355934 CAPLUS
DOCUMENT NUMBER: 143:322911
TITLE: Melanoma differentiation-associated gene-7 protein physically associates with the double-stranded RNA-activated protein kinase PKR
AUTHOR(S): Pataer, Abujiang; Vorburger, Stephan A.; Chada, Sunil; Balachandran, Siddharth; Barber, Glen N.; Roth, Jack A.; Hunt, Kelly K.; Swisher, Stephen G.
CORPORATE SOURCE: Department of Thoracic and Cardiovascular Surgery, University of Texas M. D. Anderson Cancer Center, Houston, TX, 77030, USA
SOURCE: Molecular Therapy (2005), 11(5), 717-723
CODEN: MTOHCK; ISSN: 1525-0016
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The authors previously reported that adenoviral-mediated overexpression of the melanoma differentiation-associated gene-7 (Ad-mda7; approved gene symbol IL24) leads to the rapid **induction** of PKR and activation of its downstream targets, resulting in **apoptosis induction** in human lung cancer cells. To evaluate the mechanism by which Ad-mda7 activates PKR, the authors studied the interaction between MDA-7 and PKR proteins. Following Ad-mda7 transduction of lung cancer cells, intracellular and extracellular MDA-7 protein was generated, leading to dose- and time-dependent PKR induction. Purified MDA-7 **protein administered** extracellularly did not **induce** PKR or **apoptosis**, suggesting that Ad-mda7-mediated PKR activation and **apoptosis** were not dependent on extracellular MDA-7 protein. Following Ad-mda7 transduction, RT-PCR demonstrated no increase in PKR mRNA levels despite increased levels of PKR protein, suggesting posttranscriptional regulation of PKR by MDA-7. Immunofluorescence and coimmunopptn. studies demonstrated that MDA-7 protein phys. interacts with

PKR. Transduction of PKR+/+ and PKR-/- transformed MEFs with Ad-mda7 demonstrated phosphorylated MDA-7 and PKR proteins in the lysates of PKR+/+ but not PKR-/- cells. These findings identify the first binding partner for MDA-7 and suggest that direct interaction between PKR and MDA-7 may be important for PKR activation and **apoptosis induction**, possibly through MDA-7 phosphorylation or activation of other downstream targets.

REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L46 ANSWER 9 OF 58 DRUGU COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2005-22224 DRUGU P T G
TITLE: Transmembrane delivery of protein and peptide drugs by TAT-mediated transduction in the treatment of cancer.
AUTHOR: Wadia J S; Dowdy S F
CORPORATE SOURCE: Univ.California
LOCATION: La Jolla, CA, USA
SOURCE: Adv.Drug Delivery Rev. (57, No. 4, 579-96, 2005) 121 Ref.
CODEN: ADDREP ISSN: 0169-409X
AVAIL. OF DOC.: Howard Hughes Medical Inst., and Dept. of Cellular and Molecular Med., Univ. of California San Diego School of Medicine, La Jolla, California 92093- 0686, U.S.A. (S.F.D.). (e-mail: sdowdy@ucsd.edu).
LANGUAGE: English
DOCUMENT TYPE: Journal
FIELD AVAIL.: AB; LA; CT
FILE SEGMENT: Literature
AN 2005-22224 DRUGU P T G
AB The transmembrane delivery of protein and peptide drugs by TAT-mediated transduction in the treatment of cancer is reviewed. The development of TAT transduction is described. The mechanism of TAT-mediated delivery into cells is explained. The delivery of anti-cancer therapies using transduction (p53 suppressor, cell cycle regulation, pro-apoptotic Smac peptide, Bcl-2 family, HER-2 pathway, von Hippel-Lindau (VHL) tumor suppressor peptide, TAT-ODD-caspase-3, TAT-merlin tumor suppressor, TAT-MAK19, dendritic cell vaccines, and phospholipase C-gamma1-(SH2)2-TAT) is discussed. TAT-mediated transduction for protein and **peptide delivery** may represent the next paradigm in out ability to modulate cell function and offers a unique avenue for the treatment of disease.
ABEX Intravitreal injection of TAT-N-terminal p53 peptide bypasses HDM2 regulation of p53 and **induces** rapid accumulation of p53, activation of apoptotic genes, and preferential killing of tumor cells. Genetic alteration of proto-oncogenes, tumor suppressor genes, and DNA damage repair genes often affect negative control of G1 phase cell cycle regulation. P16INK4 binds to monomeric Cdk4 or Cdk6, and leads to a G0/G1 phase cell cycle arrest. Overexpressions of p21 and p27 leads to an early G1 phase cell cycle arrest. Inhibitors of **apoptosis** proteins regulate intracellular caspase activity by blocking caspase-active sites. A region from the proapoptotic proteins called the BH3 domain antagonizes the anti-apoptotic function of Bcl-X(L), and by conjugating the BH3 domain peptide to the transduction domain from antennapedia. However, antennapedia transduction domain or BH3 peptides alone as well as a point mutant Ant-BH3-L78A peptide fail to initiate **apoptosis**. Modulation of HER-2 signal transduction pathways is a logical strategy for treating HER-2 overexpressing cancers. The VHL tumor suppressor gene is functionally inactivatedin renal cell carcinomas. Administration of TAT-oxygen-dependent degradation-caspase-3 wild-type inhibits tumor growth and reduce tumor mass. TAT-merlin isoform 1 reverses the cytoskeletal defects due to loss of merlin and controlled tumor growth. TAT-MAK19 decreases DNA synthesis followed by cell death. The transduced dendritic cells **induce** cytotoxic T lymphocyte activity, and results in partial tumor regression. The adaptor protein phospholipase C-gamma1 is an appropriate target for limiting the formation of metastasis. (MMF/NLV)

L46 ANSWER 10 OF 58 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2005:413591 CAPLUS
DOCUMENT NUMBER: 143:23619

TITLE: Early apoptotic and late necrotic components associated with altered Ca2+ homeostasis in a peptide-delivery model of polyglutamine-induced neuronal death
AUTHOR(S): Suzuki, Mari; Koike, Tatsuro
CORPORATE SOURCE: Molecular Neurobiology Laboratory, Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo, Japan
SOURCE: Journal of Neuroscience Research (2005), 80(4), 549-561
CODEN: JNREDK; ISSN: 0360-4012
PUBLISHER: Wiley-Liss, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The mechanisms by which polyglutamine expansion causes common features of neuronal death remain unclear. Here we describe an approach for delivering polyglutamine expansions directly into cultured sympathetic neurons. Glutamine (Q) residues (n = 10, 22, 30) were conjugated with a peptide possessing translocation properties across plasma membranes (PDP) and a nuclear localization signal (NLS). These peptides were rapidly incorporated into sympathetic neurons and showed neurotoxicity in a length- and dose-dependent manner. A robust induction of c-jun and cyclin D1 occurred following treatment with PDP-Q22-NLS. Enhanced c-Jun phosphorylation showed c-Jun N-terminal kinase (JNK) activation. Coincidentally, TrkA tyrosine phosphorylation was decreased in association with loss of phospho-Akt, the downstream target of PI-3 kinase. Despite such proapoptotic signals, neither release of cytochrome c from mitochondria nor caspase-3/7 activation was detected. TdT-mediated dUTP nick-end labeling-pos. nuclear condensation, but no fragmentation, occurred. At 24 h of treatment, cytoplasmic Ca2+ levels began to become elevated, and the cellular level of ATP was decreased. Cytoplasmic Ca2+ responses to KCl depolarization displayed a delayed recovery, providing evidence for lack of Ca2+ homeostasis. The neurons became committed to death at about 36 h when mitochondrial Ca2+ uptake declined concurrently with loss of mitochondrial membrane potential. Collectively, these results show that, despite induction of early apoptotic signals, nonapoptotic neuronal cell death occurred via perturbed Ca2+ homeostasis and suggest that mitochondrial permeability transition may play important roles in this model of neuronal death.

REFERENCE COUNT: 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L46 ANSWER 11 OF 58 DRUGU COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2005-22222 DRUGU G
TITLE: Membrane-permeable arginine-rich peptides and the translocation mechanisms.
AUTHOR: Futaki S
CORPORATE SOURCE: Univ.Kyoto; Japan-Sci.Technol.
LOCATION: Kyoto; Saitama, Jap.
SOURCE: Adv.Drug Delivery Rev. (57, No. 4, 547-58, 2005) 3 Fig. 2 Tab. 61 Ref.
CODEN: ADDREP ISSN: 0169-409X
AVAIL. OF DOC.: Institute for Chemical Research, Kyoto University, Uji Kyoto 611-0011, Japan. (e-mail: futaki@scl.Kyoto-u.ac.jp).
LANGUAGE: English
DOCUMENT TYPE: Journal
FIELD AVAIL.: AB; LA; CT
FILE SEGMENT: Literature
AN 2005-22222 DRUGU G

AB The structural characteristics of membrane-permeable arginine-rich peptides are reviewed. The translocation and internalization mechanisms of Tat and Tat-related peptides, arginine-rich RNA/DNA binding peptides, oligoarginine/oligoguanidino peptides, and acylated arginine-rich peptides are discussed. The effect of a macropinocytosis inhibitor, ethylisopropylamiloride (EIPA), as well as a Na+/H+ exchange protein inhibitor on the uptake of octaarginine peptide by HeLa cells is mentioned. Findings show that not only the Tat peptide, but also many arginine-rich peptides can be carrier vectors.
ABEX The HIV-1 Tat is a protein composed of 86 amino acids, which binds to the

trans-acting response element of the viral RNA to transactivate the viral promoter. The sequence of the Tat (48-60) peptide contains 6 arginines and 2 lysines within 13 amino acid residues, which are responsible for the hydrophilic and basic nature of the peptide. Internalization of nuclear localization sequence (NLS) peptides derived from nucleoplasmin, which is rich in lysine, is less efficient compared to that of the Tat peptide. The peptides showing the highest class of internalization efficiency include the RNA-binding segments derived from HIV-1 Rev, the flock house virus coat, the brome mosaic virus Gag, and the human T-cell lymphotrophic virus-II Rex protein.The peptide segment responsible for the translocation of Tat (residues 48-60) and penetratin is located in the middle of the **protein**. **Delivery** of an **apoptosis-inducing** peptide is also accomplished using the R8 peptide as a carrier peptide and the conjugate is shown to **induce apoptosis** in HeLa cells. Internalization of most of the spacer-containing derivatives is more efficient than heptaarginine itself. Oligomers of beta-amino acids bearing guanidine moieties are able to translocated through cell membranes. The uptake of the octaarginine peptide by HeLa cells is suppressed by a EIPA, as well as a Na+/H+ exchange protein inhibitor. Even though the total cellular uptake of the R15 peptide is considerably higher than the other peptides, the amount of the peptides in the cytosol is almost the same. (MMF/NLV)

L46 ANSWER 12 OF 58 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2005:186271 CAPLUS
TITLE: Synthetic design of therapeutic bacteria
AUTHOR(S): Voigt, Christopher
CORPORATE SOURCE: Department of Pharmaceutical Chemistry, UCSF, San Francisco, CA, 94107, USA
SOURCE: Abstracts of Papers, 229th ACS National Meeting, San Diego, CA, United States, March 13-17, 2005 (2005), BIOT-027. American Chemical Society: Washington, D. C.
CODEN: 69GQMP
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB Pathogenic bacteria contain numerous components that are useful for therapeutic applications in synthetic biol. They are capable of recognizing unique surface features to identify and invade specific host cells. Once bound, they engage in biochem. crosstalk with the host by using a needle-like appendage to pump effectors' across the membrane. These effectors interact with signaling proteins and can **induce** a variety of host responses, including cytokine production, actin reorganization, and **induction** of **apoptosis**. Furthermore, pathogens are able to survive host stresses and induce processes that facilitate their transfer between organs. Our lab focuses on reengineering these systems to utilize the pathogen's capabilities to survive in a host, recognize cell types, communicate with a host cell, and **deliver protein** therapeutics.

L46 ANSWER 13 OF 58 IFIPAT COPYRIGHT 2005 IFI on STN
AN 10685649 IFIPAT;IFIUDB;IFICDB
TITLE: CYTOTOXIC PEPTIDES AND PEPTIDOMIMETICS BASED THEREON, AND METHODS FOR USE THEREOF
INVENTOR(S): Bredesen; Dale, Novato, CA, US
PATENT ASSIGNEE(S): Unassigned
PATENT ASSIGNEE PROBABLE: Buck Inst (Probable)
AGENT: FOLEY & LARDNER, P.O. BOX 80278, SAN DIEGO, CA, 92138-0278, US

	NUMBER	PK	DATE	
PATENT INFORMATION:	US 2004192889	A1	20040930	
APPLICATION INFORMATION:	US 2002-472812		20020329	
	WO 2002-US9649		20020329	
			20020329	PCT 371 date
			20020329	PCT 102(e) date

	NUMBER	DATE

PRIORITY APPLN. INFO.:	US 2001-280515P	20010329 (Provisional)
	US 2001-281050P	20010402 (Provisional)
FAMILY INFORMATION:	US 2004192889	20040930
DOCUMENT TYPE:	Utility	
	Patent Application - First Publication	
FILE SEGMENT:	CHEMICAL	
	APPLICATION	

PARENT CASE DATA:

This application claims the benefit of U.S. Provisional Application No. 60/280,615, filed Mar. 30, 2001, and U.S. Provisional Application No. 60/281,050, filed Apr. 2, 2000, the contents of both of which are hereby incorporated by reference herein in their entirety.

NUMBER OF CLAIMS: 22 11 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 collectively illustrates APP interaction with and cleavage by caspases in cultured cells. In FIG. 1a, APP (a type1 integral membrane glycoprotein) is illustrated, as are fragments produced by caspase cleavage in the intracellular region of APP; and the antibodies used herein. Cleavage of APP at the caspase consensus site, VEVD/A, after the aspartic acid, is predicted to yield an N-terminal protein of 664 amino acids (APP Delta C31) and a C-terminal peptide of 31 amino acids (C31) , which contains the APP internalization signal NPTY (SEQ ID NO:5). 5A3 and 1G7 are monoclonal antibodies against the same extracellular region of APP (mixed together to detect the fulllength APP and APP Delta C31); CT15 is a polyclonal rabbit antibody against the C-terminal 15 amino acids of APP; 26D6 is a monoclonal antibody against A beta 1-12; and alpha-1 is a polyclonal antibody against APP amino acids 649-664.

FIG. 1b shows that APP interacts with caspases. APP was coimmunoprecipitated with caspases-6,-7,-8 and -9 from 293T cells co-transfected with APP and the respective caspases tested. Catalytic mutant caspases with the active site cysteine mutated to alanine were used so that co-immunoprecipitations could be done without cell death **induction**. Monoclonal antiFLAG M2 was used for the co-immunoprecipitation of FLAG-tagged caspases. Western blot analysis used monoclonal antibody (5A3/ 1G7) for APP. Lane 5 shows that caspase-8 does not interact with APP Delta C31. Lane 7 shows cells transfected with APP and immunoprecipitated and probed with monoclonal antibody 5A3/1G7 as a positive control. Quantitative densitometry analysis showed that C8 had an intensity about 200% of that of the other caspases tested (C6, 1.2; C7, 1.0; C8, 2.2; C9, 1.1).

FIG. 1c shows that APP is cleaved in 293T cells co-expressing APP and caspase-8. Cell lysate samples were immunodepleted with CT15 (Immunodep CT), then immunoprecipitated with either the mixture of monoclonal mouse antibodies 5A3 and 1G7 (MAb) or CT15 (CT). After immunodepletion, a principal C-terminal truncated species is evident (lane 1); imununodepletion removes most of the full-length APP species (lane 2). The faint bands migrating at a higher molecular weight (lanes 1 and 3) represent endogenous APP751 present in 293T cells.

FIG. 2 collectively illustrates the results of cell death and viability assays in cultured cells expressing various APP and Cterminal fragment (CTF) constructs. FIG. 2a shows cell death in N2a cells transfected with various constructs. Expression of C31 increases cell death compared with control ($P<0.001$ by oneway ANOVA ($P<0.0001$; $F=44.838$), post-hoc Tukey-Kramer). Transfection of cells with APP ($P<0.001$) or V642F ($P<0.001$) also causes significant cell death compared with control.

FIG. 2b shows cell death in 293T cells when various constructs co-expressed with caspase-7 or -8. Caspase-8 is significantly more toxic when co-expressed with APP in 293T cells than caspase-8 or APP expressed alone ($P<0.001$ by two-way ANOVA ($P<0.00001$; $F=186.9$), post-hoc Tukey HSD).

FIG. 2c shows the viability of 293T cells in which **apoptosis** was ***induced*** with tamoxifen in the presence of various constructs. C100 causes more cell death than APP ($P<0.001$, one-way ANOVA ($P<0.0001$; $F=157.58$), post-hoc Tukey-Kramer) but slightly less cell death than C31 ($P<0.05$). C100-D644A abolishes all of the cytotoxic effects of C100, compared with mock transfection with pcDNA3 ($P>0.05$).

FIG. 3 illustrates iin vivo caspase-9 activation in AD and control brains. Crude synaptosomal preparations were iumnunoprecipitated with a polyclonal

antibody against caspase9, followed by western blot analysis with an activationspecific antibody against caspase-9. Lane 1 shows Hela cells transfected with caspase-9 and treated with the pan-caspase inhibitor zVAD.fmk. Lane 2 shows caspase-9 transfected staurosporine-treated Hela cells. Lane 3 shows active recombinant caspase-9. In all five AD patients and one neurologically affected non-AD control patient (with normalpressure hydrocephalus and dementia) there are activated caspase-9 p10 fragments(*).
FIG. 4 demonstrates APP C31 toxicity to both neurons and glial cells in primary hippocampal cultures. Cultures were treated with various concentrations of the penetration peptide conjugated to C31 (DP-APPC31) or 10 mu M of DP, immunostained 24 hours later with antibodies specific for the neuronal marker NeuN or the glial marker GFAP. Relative area values for NeuN and GFAP immunoreactivity were obtained by image analysis using the Simple PCI software (Compix, Inc., Philadelphia). The broad spectrum caspase inhibitor BAF blocked the toxicity of the DPAPPC31 conjugate at 10 mu M.
FIG. 5 demonstrates that transduction of APP C31 **induces** overall cell death in hippocampal cultures. Primary hippocampal cultures were transduced with the penetratili **peptide** (**delivery peptide** DP) or the DP-APPC31 conjugate. at various concentrations and then assayed for viability 36 hours later by the MTT assay.
FIG. 6 collectively shows APP C31 **induced** cell death in primary hippocampal culture, FIG. 6a measures viability of the cultures by the trypan blue exclusion method thirty hours after transduction with the peptides. FIG. 6b measures condensed, fragmented nuclei by staining cultures with 0.1 mg/ml Hoechst 33342 30 hours after transduction with the peptides.
FIG. 7 collectively shows that the C-terminal cleavage product of the APP homolog, APLP1, **induces** death in primary hippocampal cultures, in primarily neurons and not glial cells. FIG. 7a shows cultures treated with increasing concentrations of DPAPLP1C31 peptide in the presence or in the absence of the caspase inhibitor, BAF. Twenty-four hours later, the cultures were immunostained with antibodies specific for NeuN (light) and GFAP (dark). FIG. 7b shows primary hippocampal cultures transduced with the indicated concentrations of DP-APLP1C31 or DP alone. Thirty hours later, the viability of the cultures was determined by the trypan blue exclusion method.

AB In accordance with the present invention, it has been discovered that the beta -amyloid precursor protein (APP), and two APPlike proteins (APLP1 and APLP2) are proteolytically cleaved by caspases in the C terminus to generate an approximately 31 amino acid peptide. It has been further discovered that the resultant C-terminal peptide is a potent inducer of apoptosis. Both caspase-cleaved APP and activated caspase-9 is present in brains of Alzheimer's disease patients but not in control brains. These findings indicate that caspase cleavage of APP and APP-like proteins leads to the generation of apoptotic peptides, which may contribute to the neuronal death associated with Alzheimer's disease. Accordingly, there are provided compositions and methods for modulating apoptosis.

CLMN 22 11 Figure(s).

FIG. 1 collectively illustrates APP interaction with and cleavage by caspases in cultured cells. In FIG. 1a, APP (a type1 integral membrane glycoprotein) is illustrated, as are fragments produced by caspase cleavage in the intracellular region of APP; and the antibodies used herein. Cleavage of APP at the caspase consensus site, VEVD/A, after the aspartic acid, is predicted to yield an N-terminal protein of 664 amino acids (APP Delta C31) and a C-terminal peptide of 31 amino acids (C31) , which contains the APP internalization signal NPTY (SEQ ID NO:5). 5A3 and 1G7 are monoclonal antibodies against the same extracellular region of APP (mixed together to detect the fulllength APP and APP Delta C31); CT15 is a polyclonal rabbit antibody against the C-terminal 15 amino acids of APP; 26D6 is a monoclonal antibody against A beta 1-12; and alpha-1 is a polyclonal antibody against APP amino acids 649-664.
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FIG. 5 demonstrates that transduction of APP C31 **induces** overall cell death in hippocampal cultures. Primary hippocampal cultures were transduced with the penetratili **peptide** (**delivery peptide** DP) or the DP-APPC31 conjugate. at various concentrations and then assayed for viability 36 hours later by the MTT assay.

FIG. 6 collectively shows APP C31 **induced** cell death in primary hippocampal culture, FIG. 6a measures viability of the cultures by the trypan blue exclusion method thirty hours after transduction with the peptides. FIG. 6b measures condensed, fragmented nuclei by staining cultures with 0.1 mg/ml Hoechst 33342 30 hours after transduction with the peptides.

FIG. 7 collectively shows that the C-terminal cleavage product of the APP homolog, APLP1, **induces** death in primary hippocampal cultures, in primarily neurons and not glial cells. FIG. 7a shows cultures treated with increasing concentrations of DPAPLP1C31 peptide in the presence or in the absence of the caspase inhibitor, BAF. Twenty-four hours later, the cultures were immunostained with antibodies specific for NeuN (light) and GFAP (dark). FIG. 7b shows primary hippocampal cultures transduced with the indicated concentrations of DP-APLP1C31 or DP alone. Thirty hours later, the viability of the cultures was determined by the trypan blue exclusion method.

L46 ANSWER 14 OF 58 IFIPAT COPYRIGHT 2005 IFI on STN

AN	10556572 IFIPAT;IFIUDB;IFICDB
TITLE:	METHODS FOR INHIBITION OF ANGIOGENESIS; BY ADMINISTERING AN ORGANIC PEPTIDOMIMETIC VIBRONECTIN RECEPTOR ALPHA V BETA 3 ANTAGONIST OF GIVEN STRUCTURES
INVENTOR(S):	Brooks; Peter C., Carmel, NY, US Cheresh; David A., Encinitas, CA, US
PATENT ASSIGNEE(S):	The Scripps Research Institute, US
AGENT:	OLSON & HIERL, LTD. 36th Floor, 20 North Wacker Drive, Chicago, IL, 60606, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2004063790	A1	20040401
APPLICATION INFORMATION:	US 2003-402212		20030328

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
Section 371 PCT Filing OF:	WO 1997-US9158	19970530	PENDING
CONTINUATION OF:	US 1999-194468	19990323	6500924
CONTINUATION-IN-PART OF:	US 2002-115223	20020402	

	NUMBER	DATE	
PRIORITY APPLN. INFO.:	US 1996-15869P	19960531	(Provisional)
	US 1996-18773P	19960531	(Provisional)
FAMILY INFORMATION:	US 2004063790	20040401	
	US 6500924		
DOCUMENT TYPE:	Utility Patent Application - First Publication		
FILE SEGMENT:	CHEMICAL APPLICATION		
OTHER SOURCE:	CA 140:281370		

GOVERNMENT INTEREST:
(0002) This invention was made with government support under Contract Nos. CA50826, CA45726, HL54444, T32 AI07244-11 and F32 CA72192 by the National Institutes of Health. The government has certain rights in the invention.

PARENT CASE DATA:

This application is a continuation-in-part of U.S. application for patent Ser. No. 10/115,223, filed on Apr. 2, 2002, which is a continuation of U.S. application for patent Ser. No. 09/194, 468, filed on Mar. 23, 1999, now U.S. Pat. No. 6,500,924, which is a U.S. National Phase application of PCT/US97/09158, filed on May 30, 1997, which claims priority from U.S. Provisional Application Serial No. 60/018,773, filed on May 31, 1996 and U. S. Provisional Application Serial No. 60/015,869, filed on May 31, 1996, the disclosures of which are incorporated herein by reference.

NUMBER OF CLAIMS: 41 23 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 illustrates the quantification in a bar graph of the relative expression of alpha v beta 3 and beta 1 in untreated and bFGF treated 10 day old CAMs. The mean fluorescence intensity is plotted on the Y-axis with the integrin profiles plotted on the X-axis.

FIG. 2 illustrates the quantification of the number of vessels entering a tumor in a CAM preparation. The graph shows the number of vessels as plotted on the Y-axis resulting from topical application of either CSAT (anti-beta 1), LM609 (antialpha v beta 3) or P3G2 (anti-alpha v beta 5).

FIGS. 3A-3D illustrate a comparison between wet tumor weights 7 days following treatment and initial tumor weights. Each bar represents the mean +-S.E. of 5-10 tumors per group. Tumors were derived from human melanoma (M21-L) (FIG. 3A), pancreatic carcinoma (Fg) (FIG. 3B), lung carcinoma (UCLAP-3) (FIG. 3C), and laryngeal carcinoma (HEp3) (FIG. 3D) CAM preparations and treated intravenously with PBS, CSAT (anti-beta 1), or LM609 (anti-alpha v beta 3). The

graphs show the tumor weight as plotted on the Y-axis resulting from intravenous application of either CSAT (anti-beta 1), LM609 (anti-alpha v beta 3) or PBS as indicated on the X-axis.

FIG. 4 represents a flow chart of how the in vivo mouse:human chimeric mouse model was generated. A portion of skin from a SCID mouse was replaced with human neonatal foreskin and allowed to heal for 4 weeks. After the graft had healed, the human foreskin was inoculated with human tumor cells. During the following 4 week period, a measurable tumor was established which comprised a human tumor with human vasculature growing from the human skin into the human tumor.

FIG. 5 illustrates the percent of **apoptosis** of cells derived from mab-treated and peptide-treated CAMs and stained with Apop Tag as determined by FACS analysis. The striped and stippled bars represent cells from embryos treated 24 hours and 48 hours prior to the assay, respectively. Each bar represents the mean +-S.E. of three replicates. CAMs were treated mAb LM609 (antialpha v beta 3), or CSAT (anti-beta 1), or PBS. CAMs were also treated with cyclic peptide 66203 (cyclo-RGDfV, indicated as Peptide 203) or control cyclic peptide 69601 (cyclo-PADfV, indicated as Peptide 601).

FIG. 6 shows the result of a inhibition of cell attachment assay with peptide 85189. The effects of the peptide antagonist was assessed over a dosage range of 0.001 to 100 uM as plotted on the X-axis. Cell attachment is plotted on the Y-axis measured at an optical density (O.D.) of 600 nm. Cell attachment was measured on vitronectin-(broken lines) versus laminin-(solid lines) coated surfaces.

FIGS. 7A-7D show the consecutive cDNA sequence of chicken MMP-2 along with the deduced amino acid sequence shown on the second line. The third and fourth lines respectively show the deduced amino acid sequence of human and mouse MMP-2. The chicken cDNA sequence is listed in SEQ ID NO: 29 along with the encoded amino acid sequence that is also presented separately as SEQ ID NO: 30. The numbering of the first nucleotide of the 5' untranslated region and the region encoding the proenzyme sequence shown in FIG. 7A as a negative number is actually presented as number 1 in Sequence Listing making the latter appear longer than the FIGS.; however, the nucleotide sequence is the FIGS. is identical in length and sequence to that as presented in the listing with the exception of the numbering. Accordingly, references to nucleotide position for chicken or human MMP-2 in the specification, such as in primers for use in amplifying MMP-2 fragments, are based on the nucleotide position as indicated in the FIGS. and not as listed in the Sequence Listing.

FIG. 8 shows the results in bar-graph form of a solid-phase receptor binding assay of iodinated MMP-2 to bind to alpha v beta 3 with and without the presence of inhibitors. The data is plotted as bound CPM on the Y-axis against the various potential inhibitors and controls.

FIG. 9 shows the specificity of chicken-derived MMP-2 compositions for either the integrin receptors alpha v beta 3 and alpha IIb beta 3 in the presence of MMP-2 inhibitors.

FIGS. 10 and 11 both illustrate in bar graph form the angiogenic index (a measurement of branch points) of the effects of chicken MMP-2(410-637) GST fusion protein (labeled CTMMP-2) versus control (RAP-GST or GST-PAP) on bFGF-treated CAMs. Angiogenic index is plotted on the Y-axis against the separate treatments on the X-axis.

FIG. 12 shows the effects of peptides and organic compounds on bFGF-***induced*** angiogenesis as measured by the effect on branch points plotted on the Y-axis against the various treatments on the X-axis, including bFGF alone, and bFGF-treated CAMs with peptides 69601 or 66203 and organic componds 96112, 96113 and 96229.

FIG. 13 graphically shows the dose response of peptide 85189 on inhibiting bFGF-**induced** angiogenesis where the number of branch points are plotted on the Y-axis against the amount of **peptide** ***administered*** to the embryo on the X-axis.

FIG. 14 shows the inhibitory activity of peptides 66203 (labeled 203) and 85189 (labeled 189) in bFGF-**induced** angiogenesis in the CAM assay. Controls included no peptide in bFGF-treated CAMS and peptide 69601 (labeled 601).

FIGS. 15, 16 and 17 respectively show the reduction in tumor weight for UCLAP-3, M21-L and FgM tumors following intravenous exposure to control peptide 69601 and antagonist 85189. The data is plotted with tumor weight on the Y-axis against the peptide treatments on the X-axis.

FIG. 18 illustrates the effect of peptides and antibodies on melanoma tumor growth in the chimeric mouse:human model. The peptides assessed included control 69601 (labeled 601) and antagonist 85189 (labeled 189). The antibody

tested was LM609. Tumor volume in mm3 is plotted on the Y-axis against the various treatments on the X-axis.
FIGS. 19A and 19B respectively show the effect of antagonist 85189 (labeled 189) compared to control peptide 69601 (labeled 601) in reducing the volume and wet weight of M21L tumors over a dosage range of 10, 50 and 250 mu g/injection.
FIGS. 20A and 20B show the effectiveness of antagonist peptide 85189 (labeled 189 with a solid line and filled circles) against control peptide 69601 (labeled 601 on a dotted line and open squares) at inhibiting M21L tumor volume in the mouse:human model with two different treatment regimens. Tumor volume in mm3 is plotted on the Y-axis against days on the Xaxis.
FIGS. 21 through 25 schematically illustrate the various chemical syntheses of organic molecule alpha v beta 3 antagonists.
FIGS. 26 and 27 show the effects of various organic molecules on bFGF-***induced*** angiogenesis in a CAM assay. Branch points are plotted on the Y-axis against the various compounds used at 250 mu g/ml on the X-axis in FIG. 26 and 100 mu g/ml in FIG. 27.
FIGS. 28 through 31 illustrate examples of organic peptidomimetic Compounds I(a) through I(r), corresponding to general formula (I), which are useful in the methods of the present invention.
FIG. 32 graphically illustrates the inhibitory effect of Compound I(e) of the invention in chick CAM angiogenesis inhibition assay.
FIG. 33 graphically depicts the inhibitory effect of Compound I(f) of the invention in a chick CAM angiogenesis inhibition assay.
FIG. 34 graphically illustrates the effects of Compound I(d) on M21-L tumor growth in athymic Wehi mice at concentrations of Compound I(d) ranging from about 3 mg/Kg/day to about 90 mg/Kg/ day.

AB The present invention describes methods for inhibition angiogenesis in tissues using organic peptidomimetic alpha v beta 3 antagonists, and particularly for inhibiting angiogenesis in inflamed tissues and in tumor tissues and metastases using therapeutic compositions containing alpha v beta 3 antagonists. The antagonists are organic compounds having a basic group and an acidic group spaced from one another by a distance in the range of about 10 Angstroms to about 100 Angstroms, as described in detail herein.

CLMN 41 23 Figure(s).
FIG. 1 illustrates the quantification in a bar graph of the relative expression of alpha v beta 3 and beta 1 in untreated and bFGF treated 10 day old CAMs. The mean fluorescence intensity is plotted on the Y-axis with the integrin profiles plotted on the X-axis.
FIG. 2 illustrates the quantification of the number of vessels entering a tumor in a CAM preparation. The graph shows the number of vessels as plotted on the Y-axis resulting from topical application of either CSAT (anti-beta 1), LM609 (antialpha v beta 3) or P3G2 (anti-alpha v beta 5).
FIGS. 3A-3D illustrate a comparison between wet tumor weights 7 days following treatment and initial tumor weights. Each bar represents the mean +-S.E. of 5-10 tumors per group. Tumors were derived from human melanoma (M21-L) (FIG. 3A), pancreatic carcinoma (Fg) (FIG. 3B), lung carcinoma (UCLAP-3) (FIG. 3C), and laryngeal carcinoma (HEp3) (FIG. 3D) CAM preparations and treated intravenously with PBS, CSAT (anti-beta 1), or LM609 (anti-alpha v beta 3). The graphs show the tumor weight as plotted on the Y-axis resulting from intravenous application of either CSAT (anti-beta 1), LM609 (anti-alpha v beta 3) or PBS as indicated on the X-axis.
FIG. 4 represents a flow chart of how the in vivo mouse:human chimeric mouse model was generated. A portion of skin from a SCID mouse was replaced with human neonatal foreskin and allowed to heal for 4 weeks. After the graft had healed, the human foreskin was inoculated with human tumor cells. During the following 4 week period, a measurable tumor was established which comprised a human tumor with human vasculature growing from the human skin into the human tumor.
FIG. 5 illustrates the percent of **apoptosis** of cells derived from mab-treated and peptide-treated CAMs and stained with Apop Tag as determined by FACS analysis. The striped and stippled bars represent cells from embryos treated 24 hours and 48 hours prior to the assay, respectively. Each bar represents the mean +-S.E. of three replicates. CAMs were treated mAb LM609 (antialpha v beta 3), or CSAT (anti-beta 1), or PBS. CAMs were also treated with cyclic peptide 66203 (cyclo-RGDfV, indicated as Peptide 203) or control cyclic peptide 69601 (cyclo-PADfV, indicated as Peptide 601).

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FIGS. 7A-7D show the consecutive cDNA sequence of chicken MMP-2 along with the deduced amino acid sequence shown on the second line. The third and fourth lines respectively show the deduced amino acid sequence of human and mouse MMP-2. The chicken cDNA sequence is listed in SEQ ID NO: 29 along with the encoded amino acid sequence that is also presented separately as SEQ ID NO: 30. The numbering of the first nucleotide of the 5' untranslated region and the region encoding the proenzyme sequence shown in FIG. 7A as a negative number is actually presented as number 1 in Sequence Listing making the latter appear longer than the FIGS.; however, the nucleotide sequence is the FIGS. is identical in length and sequence to that as presented in the listing with the exception of the numbering. Accordingly, references to nucleotide position for chicken or human MMP-2 in the specification, such as in primers for use in amplifying MMP-2 fragments, are based on the nucleotide position as indicated in the FIGS. and not as listed in the Sequence Listing.

FIG. 8 shows the results in bar-graph form of a solid-phase receptor binding assay of iodinated MMP-2 to bind to alpha v beta 3 with and without the presence of inhibitors. The data is plotted as bound CPM on the Y-axis against the various potential inhibitors and controls.

FIG. 9 shows the specificity of chicken-derived MMP-2 compositions for either the integrin receptors alpha v beta 3 and alpha IIb beta 3 in the presence of MMP-2 inhibitors.

FIGS. 10 and 11 both illustrate in bar graph form the angiogenic index (a measurement of branch points) of the effects of chicken MMP-2(410-637) GST fusion protein (labeled CTMMP-2) versus control (RAP-GST or GST-PAP) on bFGF-treated CAMs. Angiogenic index is plotted on the Y-axis against the separate treatments on the X-axis.

FIG. 12 shows the effects of peptides and organic compounds on bFGF-**induced** angiogenesis as measured by the effect on branch points plotted on the Y-axis against the various treatments on the X-axis, including bFGF alone, and bFGF-treated CAMs with peptides 69601 or 66203 and organic componds 96112, 96113 and 96229.

FIG. 13 graphically shows the dose response of peptide 85189 on inhibiting bFGF-**induced** angiogenesis where the number of branch points are plotted on the Y-axis against the amount of **peptide administered** to the embryo on the X-axis.

FIG. 14 shows the inhibitory activity of peptides 66203 (labeled 203) and 85189 (labeled 189) in bFGF-**induced** angiogenesis in the CAM assay. Controls included no peptide in bFGF-treated CAMS and peptide 69601 (labeled 601).

FIGS. 15, 16 and 17 respectively show the reduction in tumor weight for UCLAP-3, M21-L and FgM tumors following intravenous exposure to control peptide 69601 and antagonist 85189. The data is plotted with tumor weight on the Y-axis against the peptide treatments on the X-axis.

FIG. 18 illustrates the effect of peptides and antibodies on melanoma tumor growth in the chimeric mouse:human model. The peptides assessed included control 69601 (labeled 601) and antagonist 85189 (labeled 189). The antibody tested was LM609. Tumor volume in mm3 is plotted on the Y-axis against the various treatments on the X-axis.

FIGS. 19A and 19B respectively show the effect of antagonist 85189 (labeled 189) compared to control peptide 69601 (labeled 601) in reducing the volume and wet weight of M21L tumors over a dosage range of 10, 50 and 250 mu g/injection.

FIGS. 20A and 20B show the effectiveness of antagonist peptide 85189 (labeled 189 with a solid line and filled circles) against control peptide 69601 (labeled 601 on a dotted line and open squares) at inhibiting M21L tumor volume in the mouse:human model with two different treatment regimens. Tumor volume in mm3 is plotted on the Y-axis against days on the Xaxis.

FIGS. 21 through 25 schematically illustrate the various chemical syntheses of organic molecule alpha v beta 3 antagonists.

FIGS. 26 and 27 show the effects of various organic molecules on bFGF-**induced** angiogenesis in a CAM assay. Branch points are plotted on

the Y-axis against the various compounds used at 250 mu g/ml on the X-axis in FIG. 26 and 100 mu g/ml in FIG. 27.

FIGS. 28 through 31 illustrate examples of organic peptidomimetic Compounds I(a) through I(r), corresponding to general formula (I), which are useful in the methods of the present invention.

FIG. 32 graphically illustrates the inhibitory effect of Compound I(e) of the invention in chick CAM angiogenesis inhibition assay.

FIG. 33 graphically depicts the inhibitory effect of Compound I(f) of the invention in a chick CAM angiogenesis inhibition assay.

FIG. 34 graphically illustrates the effects of Compound I(d) on M21-L tumor growth in athymic Wehi mice at concentrations of Compound I(d) ranging from about 3 mg/Kg/day to about 90 mg/Kg/ day.

L46 ANSWER 15 OF 58 USPAT2 on STN

ACCESSION NUMBER: 2004:58184 USPAT2

TITLE: Secreted protein HHTLF25

INVENTOR(S): Rosen, Craig A., Laytonsville, MD, UNITED STATES
Ruben, Steven M., Olney, MD, UNITED STATES
LaFleur, David W., Washington, DC, UNITED STATES

PATENT ASSIGNEE(S): Human Genome Sciences, Inc., Rockville, MD, UNITED STATES (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6924354	B2	20050802
APPLICATION INFO.:	US 2001-973278		20011010 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1999-227357, filed on 8 Jan 1999, Pat. No. US 6342581, issued on 29 Jan 2002 Continuation-in-part of Ser. No. WO 1998-US13684, filed on 7 Jul 1998, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-239899P	20001013 (60)
	US 1997-51926P	19970708 (60)
	US 1997-52793P	19970708 (60)
	US 1997-51925	19970708 (06)
	US 1997-51929P	19970708 (60)
	US 1997-52803P	19970708 (60)
	US 1997-52732P	19970708 (60)
	US 1997-51931P	19970708 (60)
	US 1997-51932P	19970708 (60)
	US 1997-51916P	19970708 (60)
	US 1997-51930P	19970708 (60)
	US 1997-51918P	19970708 (60)
	US 1997-51920P	19970708 (60)
	US 1997-52733P	19970708 (60)
	US 1997-52795P	19970708 (60)
	US 1997-51919P	19970708 (60)
	US 1997-51928P	19970708 (60)
	US 1997-55722P	19970818 (60)
	US 1997-55723P	19970818 (60)
	US 1997-55948P	19970818 (60)
	US 1997-55949P	19970818 (60)
	US 1997-55953P	19970818 (60)
	US 1997-55950P	19970818 (60)
	US 1997-55947P	19970818 (60)
	US 1997-55964P	19970818 (60)
	US 1997-56360P	19970818 (60)
	US 1997-55684P	19970818 (60)
	US 1997-55984P	19970818 (60)
	US 1997-55954P	19970818 (60)
	US 1997-58785P	19970912 (60)
	US 1997-58664P	19970912 (60)
	US 1997-58660P	19970912 (60)
	US 1997-58661P	19970912 (60)

DOCUMENT TYPE: Utility

FILE SEGMENT: GRANTED

PRIMARY EXAMINER: Meyers, Carla J.

ASSISTANT EXAMINER: Spiegler, Alexander H.
LEGAL REPRESENTATIVE: Human Genome Sciences, Inc.
NUMBER OF CLAIMS: 46
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 3 Drawing Figure(s); 3 Drawing Page(s)
LINE COUNT: 36245
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating diseases, disorders, and/or conditions related to these novel human secreted proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L46 ANSWER 16 OF 58 USPAT2 on STN
ACCESSION NUMBER: 2004:12959 USPAT2
TITLE: Methods and compositions for diagnosing or monitoring auto immune and chronic inflammatory diseases
INVENTOR(S): Wohlgemuth, Jay, Palo Alto, CA, UNITED STATES
Fry, Kirk, Palo Alto, CA, UNITED STATES
Woodward, Robert, Pleasanton, CA, UNITED STATES
Ly, Ngoc, San Bruno, CA, UNITED STATES
PATENT ASSIGNEE(S): Expression Diagnostics, Inc., So. San Francisco, CA, UNITED STATES (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6905827	B2	20050614
APPLICATION INFO.:	US 2002-131827		20020424 (10)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2001-6290, filed on 22 Oct 2001, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-296764P	20010608 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Martinell, James
LEGAL REPRESENTATIVE: Morrison & Foerster LLP
NUMBER OF CLAIMS: 9
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 12 Drawing Figure(s); 9 Drawing Page(s)
LINE COUNT: 57528
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods of diagnosing or monitoring an autoimmune or chronic inflammatory disease, particularly SLE in a patient by detecting the expression level of one or more genes or surrogates derived therefrom in the patient are described. Diagnostic oligonucleotides for diagnosing or monitoring chronic inflammatory disease, particularly SLE infection and kits or systems containing the same are also described.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L46 ANSWER 17 OF 58 TOXCENTER COPYRIGHT 2005 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 2004:169513 TOXCENTER
COPYRIGHT: Copyright 2005 ACS
DOCUMENT NUMBER: CA14108128667V
TITLE: Arginine carrier peptide bearing Ni(II) chelator to promote cellular uptake of histidine-tagged proteins
AUTHOR(S): Futaki, Shiroh; Niwa, Miki; Nakase, Ikuhiko; Tadokoro, Akiko; Zhang, Youjun; Nagaoka, Makoto; Wakako, Naoya; Sugiura, Yukio
CORPORATE SOURCE: Institute for Chemical Research, Kyoto University, Kyoto, 611-0011, Japan.
SOURCE: Bioconjugate Chemistry, (2004) Vol. 15, No. 3, pp. 475-481.

CODEN: BCCHES. ISSN: 1043-1802.
COUNTRY: JAPAN
DOCUMENT TYPE: Journal
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 2004:359954
LANGUAGE: English
ENTRY DATE: Entered STN: 20040803
Last Updated on STN: 20041221

AB Arginine-rich peptide-mediated **protein delivery** into living cells is a novel technol. for controlling cell functions with therapeutic potential. A novel approach for the intracellular delivery of histidine-tagged proteins was introduced where a Ni(II) chelate of octaarginine peptide bearing nitrilotriacetic acid [R8-NTA-Ni(II)] was used as a membrane-permeable carrier mol. Significant internalization of histidine-tagged enhanced green fluorescent protein (EGFP) into HeLa cells was observed by confocal microscopic observation in the presence of R8-NTA-Ni(II). Nuclear condensation characteristic in apoptotic cell death was also **induced** in the cells treated with a histidine-tagged **apoptosis-inducing** peptide [pro-apoptotic domain peptide (PAD)], indicating that the cargo mols. really went through the membrane to reach the cytosol. The **apoptosis-inducing** activity of the peptide thus delivered was compared with that of the PAD peptide covalently connected with the octaarginine peptide.

L46 ANSWER 18 OF 58 TOXCENTER COPYRIGHT 2005 ACS on STN DUPLICATE 4
ACCESSION NUMBER: 2004:237449 TOXCENTER
COPYRIGHT: Copyright 2005 ACS
DOCUMENT NUMBER: CA14212225323H
TITLE: Delivery of novel anti-cancer peptides by protein transduction domains
AUTHOR(S): Wadia, J. S.; Ezhevsky, S.; Dowdy, S. F.
CORPORATE SOURCE: Howard Hughes Medical Institute and Department of Cellular and Molecular Medicine, University of California San Diego School of Medicine, CA, USA.
SOURCE: American Pharmaceutical Review, (2004) Vol. 7, No. 3, pp. 65-69.
CODEN: APHRFS. ISSN: 1099-8012.
COUNTRY: UNITED STATES
DOCUMENT TYPE: Journal
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 2004:575670
LANGUAGE: English
ENTRY DATE: Entered STN: 20041026
Last Updated on STN: 20050405

AB A review on the basic concepts of protein transduction technol. and how this technol. can be applied to **deliver proteins** and peptides in cancer therapy. Protein transduction domain-mediated delivery of pro-apoptotic peptides, either alone or in combination therapy, is a highly promising strategy to specifically kill tumor cells while leaving normal cells unharmed. This approach has been successfully used in several cell culture studies to **induce** tumor-specific **apoptosis**. Dramatic tumor burden reduction and survival prolongation have been documented in several preclin. mouse tumor models in vivo, paving the way for imminent phase I clin. trials.

L46 ANSWER 19 OF 58 DISSABS COPYRIGHT (C) 2005 ProQuest Information and Learning Company; All Rights Reserved on STN
ACCESSION NUMBER: 2004:28480 DISSABS Order Number: AAIC814845 (not available for sale by UMI)
TITLE: Modifying T cell differentiation via protein-based delivery of signalling attenuators
AUTHOR: Veldhoen, Marc [Ph.D.]
CORPORATE SOURCE: Open University (United Kingdom) (0949)
SOURCE: Dissertation Abstracts International, (2003) Vol. 65, No. 1C, p. 106. Order No.: AAIC814845 (not available for sale by UMI).
DOCUMENT TYPE: Dissertation
FILE SEGMENT: DAI

LANGUAGE: English
ENTRY DATE: Entered STN: 20040528
Last Updated on STN: 20040528

AB In recent years, evidence has accumulated that hierarchical thresholds for proliferation and differentiation may determine the generation of various effector T cell modes. It is as yet poorly understood, however, how changes in TCR-mediated signalling and its downstream signalling cascades drive the differentiation of the naive T cell into different response modes. This project aims to study whether manipulation of the signalling pathways directly downstream of the TCR/CD28 receptor can result in distinct functional differentiation of monoclonal CD4+ T cell populations with known antigenic specificity.

To this end, two potential protein transfer mechanisms were tested for their suitability to act as inert delivery vehicles to **deliver proteins** into ex vivo naive T cells. The B subunit pentamer of enterotoxin B **induced** high levels **apoptosis** in naive T cells, which precluded its use as an inert delivery vehicle. The protein transduction domain of the HIV-1 protein Tat, however, did not influence T cell physiology and was subsequently successfully tested for its ability to transduce ex vivo T cells genetically fused to large proteins.

Two fusion partners, the kinase truncated forms of ZAP-70 and Lck, were tested for their ability to interfere with TCR-mediated signalling. The kinase-truncated form of ZAP-70 showed a concentration dependent effect on calcium mobilisation, which was reliant on the strength of CD3 crosslinking. The kinase-truncated form of Lck also altered calcium mobilisation in a concentration dependent manner, but independent of TCR strength. In addition, it was shown to influence T cell activation, to be capable of altering the signal transduction cascade after T cell priming, and to dramatically change the cytokine profile.

L46 ANSWER 20 OF 58 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:334640 CAPLUS
DOCUMENT NUMBER: 138:348684
TITLE: Methods of inducing apoptosis in hyperproliferative cells using human potassium channel modulatory protein KChAP for cancer therapy
INVENTOR(S): Brown, Arthur M.; Wible, Barbara A.
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 29 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003083249	A1	20030501	US 2001-778	20011031
WO 2003038051	A2	20030508	WO 2002-US34722	20021031
WO 2003038051	C2	20040513		
WO 2003038051	A3	20050217		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UZ, VN, YU, ZA, ZM, ZW

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 2004259791	A1	20041223	US 2004-784528	20040223
PRIORITY APPLN. INFO.:			US 2001-778 A	20011031

AB Methods of inducing apoptosis in hyperproliferative cells, particularly cancer cells are provided. Such method involves increasing the levels of a potassium channel modulatory protein in the cell. The method is based on the discovery that KChAP induces apoptosis in the prostate cancer cell line, LNCaP, which expresses both K+ currents and wild-type p53. Specifically, the infection with a recombinant adenovirus encoding KChAP

(Ad/KChAP) increases K+ efflux and reduces cell size as expected for an apoptotic volume decrease. The apoptosis inducer, staurosporine, increases endogenous KChAP levels, and LNCaP cells, 2 days after Ad/KChAP infection, show increased sensitivity to staurosporine. KChAP increases p53 levels and stimulates phosphorylation of p53 residue serine 15. Consistent with activation of p53 as a transcription factor, p21 levels are increased in infected cells. Wild-type p53 is not essential for induction of apoptosis by KChAP, however, since KChAP also induces apoptosis in DU145 cells, a prostate cancer cell line with mutant p53. Consistent with its proapoptotic properties, KChAP prevents growth of DU145 and LNCaP tumor xenografts in nude mice, indicating that infection with Ad/KChAP might represent a novel method of cancer treatment. Examples of such proteins are native KChAP protein, a biol. active variant of native KChAP protein, or a biol. active KChAP-related protein (collectively referred to hereinafter as "KChAP protein"). In one embodiment, the cells are contacted with the KChAP protein under conditions permitting uptake of the protein by the cells. In another embodiment, the cells are contacted with (i) a nucleic acid encoding the KChAP protein, and (ii) a promoter active in the cancer cell, wherein the promoter is operably linked to the region encoding the KChAP protein, under conditions permitting the uptake of the nucleic acid by the cancer cell. Methods of detecting cancerous cells in a biol. sample selected from the group consisting of a colorectal tissue sample or brain tissue sample are also provided. Such method comprises assaying for the presence of elevated levels of KChAP mRNA or KChAP protein in the sample.

L46 ANSWER 21 OF 58 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2003-697526 [66] WPIDS
CROSS REFERENCE: 2001-273309 [28]
DOC. NO. CPI: C2003-191820
TITLE: New internalizing peptides, useful for facilitating the delivery, uptake and cytoplasmic and/or nuclear transport of proteins, DNA or viruses into a target cell, for inducing apoptosis in arthritic or tumor cells, or in gene therapy.
DERWENT CLASS: B04 D16
INVENTOR(S): FRIZZEL, R; GAMBOTTO, A; GLORIOSO, J C; MAI, J C; MI, Z; ROBBINS, P D
PATENT ASSIGNEE(S): (UYPI-N) UNIV PITTSBURGH
COUNTRY COUNT: 102
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2003068942	A2	20030821	(200366)*	EN	171
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PT SD SE SI SK SL SZ TR TZ UG ZM ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW					
AU 2003216289	A1	20030904	(200428)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003068942	A2	WO 2003-US4632	20030212
AU 2003216289	A1	AU 2003-216289	20030212

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2003216289	A1 Based on	WO 2003068942

PRIORITY APPLN. INFO: US 2002-75869 20020213
AN 2003-697526 [66] WPIDS

CR 2001-273309 [28]
AB WO2003068942 A UPAB: 20040429
NOVELTY - An internalizing peptide comprising any one of 14 fully defined amino acid sequences (designated P1-P14) given in the specification and below, is new.

DETAILED DESCRIPTION - An internalizing peptide comprising:

(a) any one of 14 fully defined amino acid sequences (designated P1-P14);

(b) an amino acid sequence comprising iterations of histidine or ornithine residues, where the peptide comprises approximately 4-18 histidine or ornithine residues; or

(c) an amino acid sequence comprising iterations of histidine residues interspersed with lysine, arginine and/or ornithine residues, where the peptide comprises approximately 4-18 residues.

Met-Tyr-Arg-Pro-Pro-Ala-Ala-Asn-Val-Asp-Pro-Trp (P1)
Ser-Ser-Pro-Pro-Pro-Asp-Leu-Thr-Thr-Arg-Thr-Pro (P2)
Ala-Thr-Thr-Gln-Ser-Thr-Pro-Pro-Ala-Phe-His-Leu (P3)
Ser-Asp-Leu-Pro-His-Val-Ser-Ser-Tyr-Trp-Arg-Gly (P4)
Thr-Thr-Thr-Gln-Phe-Met-Glu-Ile-Arg-Gln-Ser-Ala (P5)
Gly-Lys-Thr-Trp-Lys-Ala-Ser-Asp-Glu-Asp-Trp-Gly (P6)
Asp-Pro-Ala-Arg-Ile-Leu-Gly-Arg-Ile-Phe-Leu (P7)
Tyr-Asn-Leu-Gln-Pro-Thr-Thr-Ser-Ala-Arg-Pro-Thr (P8)
Ser-Leu-Lys-Thr-Pro-Thr-Thr-Ser-His-Leu-Ser-Gln (P9)
Thr-Phe-Asp-Leu-Arg-Asn Asn-Thr-His Arg-Asn-Pro (P10)
Ser-Val-Ser-Val-Gly-Met-Lys-Pro-Ser-Pro-Arg-Pro (P11)
Arg-Arg-Gln Arg-Arg (P12)
Arg-Arg-Gln Arg-Arg-Gln-Arg-Arg (P13)
Arg-Arg-Gln-Arg-Arg-Gln-Arg-Arg-Gln-Arg-Arg (P14)

INDEPENDENT CLAIMS are included for:

(1) a peptide-cargo complex comprising the internalizing peptide cited above and a cargo, where the cargo is a smac peptide, smac functional variant, smac mutant peptide or smac peptidomimetic, and where the cargo is capable of **inducing apoptosis**;

(2) a method for identifying peptides capable of cellular internalization of cargo linked to it, comprising isolating internalized peptides presented by the peptide display library by linking the peptides to cargo to form a peptide-cargo complex, incubating the peptide-cargo complex with a target cell, and determining the ability of the peptide to facilitate the cellular internalization of the cargo into the target cell;

(3) an expression cassette comprising a DNA encoding a fusion protein comprising a leader sequence, a protein of interest and the internalizing peptide above;

(4) a transfer vector comprising the expression cassette;

(5) a method for inducing synovial cell death comprising administering the peptide-cargo complex to the synovial cell;

(6) a method for **inducing apoptosis** in a tumor cell comprising administering the peptide-cargo complex to the tumor cell;

(7) a method for reducing white blood cells in arthritic joints comprising administering the peptide-cargo complex to the white blood cells;

(8) a method for inhibiting apoptosis in an islet cell comprising administering the peptide-cargo complex to the islet cell;

(9) a method for delivering anti-oxidant and anti-inflammatory agents to lung epithelial cells comprising administering the peptide-cargo complex to the lung epithelial cells;

(10) a method of internalization of a peptide-cargo complex into a cell comprising administering to the cell the peptide-cargo complex and an agent that facilitates internalization;

(11) a method for internalizing a glutathione-S-transferase (GST)-fusion protein into a cell comprising administering to the cell the peptide-cargo complex and a GST fusion protein;

(12) a kit for internalizing a GST-fusion protein into a cell comprising the peptide-cargo complex;

(13) an immunogen comprising the peptide-cargo complex; and

(14) a method for eliciting an immune response in a subject comprising administering the immunogen to a target cell of the subject.

ACTIVITY - Cytostatic; Antiinflammatory; Immunomodulator; Antiarthritic.

Three mice from each group were injected with DU145 cells in each

flank. Palpable tumors developed within 3 weeks at which time the tumors were injected daily with 50 micro l of either 1 mM protein transduction domain 5 (PTD5)) or 1 mM PTD5-smac34. The injections proceeded for 10 days and tumor volume was estimated each day. Results showed that PTD5-smac34 reduced tumor size over a two-week period.

Arg-Arg-Gln-Arg-Arg-Thr-Ser-Lys-Leu-Met-Lys-Arg-Gly-Gly (PTD5)

MECHANISM OF ACTION - Gene Therapy; Vaccine.

USE - The internalizing peptides are useful for facilitating the delivery, uptake and cytoplasmic and/or nuclear transport of cargo, e.g. proteins, DNA or viruses, into a target cell. The internalizing peptides and peptide-cargo complexes are also useful for inducing apoptosis in cells (e.g. arthritic cells or tumor cells), expanding a population of stem cell or differentiated cells, stimulating the differentiation of a population of stem cells, facilitating the integration of adeno-associated virus DNA into the genome of a cell, stimulating or eliciting an immune response in a subject, facilitating the delivery of immunogens (e.g. vaccines), inhibiting the inflammatory process, protecting tissue from apoptosis or necrosis during tissue isolation prior to transplantation, facilitating transfer of proteins and peptides to the lung for the treatment of cystic fibrosis or lung inflammation, or in gene therapy.

Dwg.0/69

L46 ANSWER 22 OF 58 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2003-354586 [33] WPIDS
DOC. NO. CPI: C2003-093487
TITLE: New telomerase inhibitory peptides useful for treating or preventing cancer and other proliferative disorder, for inducing cell death or cell proliferation, and for inhibiting cell growth.
DERWENT CLASS: B04
INVENTOR(S): LI, H; LIU, J
PATENT ASSIGNEE(S): (LIHH-I) LI H; (LIUJ-I) LIU J
COUNTRY COUNT: 101
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2003024997	A1	20030327	(200333)*	EN	25
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW					
AU 2002325676	A1	20030401	(200452)		
CN 1575300	A	20050202	(200532)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003024997	A1	WO 2002-AU1263	20020913
AU 2002325676	A1	AU 2002-325676	20020913
CN 1575300	A	CN 2002-820847	20020913

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2002325676	A1 Based on	WO 2003024997

PRIORITY APPLN. INFO: AU 2001-7697 20010914
AN 2003-354586 [33] WPIDS
AB WO2003024997 A UPAB: 20030526

NOVELTY - A peptide sequence (I) encoding a telomerase inhibitory peptide (TEIPP), its functional equivalent, variant or fragment, is new.

DETAILED DESCRIPTION - A peptide sequence having the amino acid sequence GARTFRRXKRAXRLTSRVK (I) encoding a telomerase inhibitory peptide

(TEIPP), its functional equivalent, variant or fragment, is new. X = E or Q.

INDEPENDENT CLAIMS are also included for:

(1) a nucleotide sequence which encodes a TEIPP and which:

(a) encodes a peptide having an amino acid sequence (I), GARTFRREKRAERLTSRVK (II), GARTFRRQKRAQRLTSRVK (III);

(b) is capable of hybridizing to a sequence in (a);

(c) is a degenerate, as a result of the genetic code, from a nucleotide sequence in (a); or

(d) is a functional equivalent, variant or fragment of (a), (b) or (c);

(2) a composition for inhibiting telomerase comprising (I), (II), (III), a functional equivalent, variant or fragment of (I), or a combination of these;

(3) a method of inhibiting activity in a cell by administering to the cell TEIPP1, TEIPP, or their combination, functional equivalents, variants or fragments;

(4) a method of inhibiting cell growth in a cell culture by administering to the cell culture a telomerase inhibitory peptide selected from TEIPP1, TEIPP, and their combination, functional equivalents, variants or fragments;

(5) a method of inducing cell death by administering to a cell a telomerase inhibitory peptide selected TEIPP1, TEIPP, and their combination, functional equivalents, variants or fragments;

(6) a method of treating cancer or a proliferative disorder in a subject by administering a telomerase inhibitory peptide to the subject, where the telomerase inhibitory peptide includes TEIPP1, TEIPP or their functional equivalent or variant; and

(7) a method of inducing cell proliferation by inhibiting production in the cell of a telomerase inhibitory peptide consisting of TEIPP1 or TEIPP.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - Telomerase inhibitor.

USE - The peptide is useful for treating or preventing cancer and other proliferative disorder, for inducing cell death or cell proliferation, and for inhibiting cell growth.

Dwg.0/6

L46 ANSWER 23 OF 58 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN

ACCESSION NUMBER: 2003-450982 [43] WPIDS

CROSS REFERENCE: 2003-111976 [10]; 2003-393509 [37]; 2003-779129 [73]; 2004-635468 [61]; 2004-662346 [64]; 2004-748573 [73]; 2004-805123 [79]; 2004-833580 [82]; 2005-101299 [11]; 2005-132260 [14]

DOC. NO. NON-CPI: N2003-359728

DOC. NO. CPI: C2003-120012

TITLE: New POSH nucleic acids and polypeptides useful for treating viral disorders, particularly disorders caused by envelop viruses, retroid viruses and RNA viruses, including retroviruses, rhabdoviruses, lentiviruses and filoviruses.

DERWENT CLASS: B04 D16 P14 S03

INVENTOR(S): ALROY, I; BEN-AVRAHAM, D; GREENER, T; TUVIA, S

PATENT ASSIGNEE(S): (PROT-N) PROTEOLOGICS INC; (ALRO-I) ALROY I; (BENA-I) BEN-AVRAHAM D; (GREE-I) GREENER T; (TUVI-I) TUVIA S

COUNTRY COUNT: 101

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 1310552	A2	20030514	(200343)*	EN	85
R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR					
WO 2003095971	A2	20031120	(200403)	EN	
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT					

RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW

US 2004025196 A1 20040205 (200411)
AU 2002365160 A1 20031111 (200442)
JP 2005519636 W 20050707 (200545) 92

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 1310552	A2	EP 2002-257796	20021111
WO 2003095971	A2	WO 2002-US36366	20021112
US 2004025196	A1 Provisional	US 2001-345846P	20011109
	Provisional	US 2002-364530P	20020315
		US 2002-293965	20021112
AU 2002365160	A1	AU 2002-365160	20021112
JP 2005519636	W	WO 2002-US36366	20021112
		JP 2004-503917	20021112

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2002365160	A1 Based on	WO 2003095971
JP 2005519636	W Based on	WO 2003095971

PRIORITY APPLN. INFO: US 2002-364530P 20020315; US 2001-345846P 20011109; US 2002-293965 20021112

AN 2003-450982 [43] WPIDS

CR 2003-111976 [10]; 2003-393509 [37]; 2003-779129 [73]; 2004-635468 [61]; 2004-662346 [64]; 2004-748573 [73]; 2004-805123 [79]; 2004-833580 [82]; 2005-101299 [11]; 2005-132260 [14]

AB EP 1310552 A UPAB: 20050715

NOVELTY - An isolated nucleic acid encoding a polypeptide comprising four SH3 domains and at least 90 % identical to an 888 amino acid sequence (P1), given in the specification, or its complement, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an expression vector comprising the novel nucleic acid;

(2) a host cell transfected with the expression vector of (1);

(3) a purified POSH polypeptide expressed by the host cell of (2);

(4) producing a recombinant POSH polypeptide;

(5) a nucleic acid encoding a fusion protein, where the nucleic acid comprises a first and second nucleic acid sequences positioned to encode a fusion protein, where the first nucleic acid sequence is a nucleic acid cited above, and the second nucleic acid sequence encodes a heterologous polypeptide;

(6) a ribonucleic acid comprising 5-1000 consecutive nucleotides of the novel nucleic acid, which when introduced into a cell, decreases the level of POSH mRNA and/or a POSH polypeptide;

(7) a composition comprising the ribonucleic acid, and an additional component such as a liposome or an excipient;

(8) an isolated polypeptide comprising at least 9 1% sequence identity to P1, where the polypeptide optionally interacts with a POSH-AP and/or the down regulation of the polypeptide decreases viral maturation;

(9) an isolated antibody or its fragment, specifically immunoreactive with an epitope of P1 or a sequence not defined in the specification;

(10) a kit for detecting a human POSH polypeptide comprising the antibody and a detectable label for detecting the antibody;

(11) identifying an antiviral or antiapoptotic agent;

(12) inhibiting infection in a subject by administering an agent that inhibits POSH activity;

(13) a non-human transgenic animal comprising cells that contain one or more recombinant constructs of POSH gene, where the expression of the POSH gene mitigates a POSH loss of function phenotype;

(14) an isolated cell of the transgenic animal of (13);

(15) constructing the non-human transgenic animal;

(16) screening for a substance for treating viral infection;

(17) evaluating the antiviral potential of an agent, or an anti-viral activity of a test compound;
(18) inhibiting viral maturation;
(19) testing a ubiquitin-related activity of a POSH polypeptide;
(20) an assay for identifying an inhibitor of ubiquitin-related activity of a POSH polypeptide;
(21) a therapeutic composition comprising an inhibitor of POSH and an excipient; and
(22) inhibiting infection in a subject.
ACTIVITY - Virucide.
No biological data is given.
MECHANISM OF ACTION - Ligase inhibitor; Gene therapy.
USE - The nucleic acids, polypeptides, compositions and methods are useful for treating viral disorders, particularly disorders caused by envelop viruses, retroid viruses and RNA viruses, including retroviruses, rhabdoviruses, lentiviruses and filoviruses.
Dwg.0/25

L46 ANSWER 24 OF 58 IFIPAT COPYRIGHT 2005 IFI on STN

AN 10474992 IFIPAT;IFIUDB;IFICDB

TITLE: CALBINDIN-D28K PROTECTION AGAINST GLUCOCORTICOID **INDUCED** CELL DEATH; GENETIC VECTORS COMPRISING POLYNUCLEOTIDE CODES FOR VITAMIN D-DEPENDENT CALCIUM BINDING **PROTEIN**, **ADMINISTERED** FOR PREVENTION OF **APOPTOSIS** IN CELLS AND PROPHYLAXIS OF OSTEOPOROSIS

INVENTOR(S): Christakos; Sylvia, Mendham, NJ, US
PATENT ASSIGNEE(S): University of Medicine & Dentistry of New Jersey, US
AGENT: PERKINS COIE LLP, POST OFFICE BOX 1208, SEATTLE, WA, 98111-1208, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2003219421	A1	20031127
APPLICATION INFORMATION:	US 2002-155567		20020523
FAMILY INFORMATION:	US 2003219421		20031127

DOCUMENT TYPE: Utility
Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION
OTHER SOURCE: CA 139:394903

GOVERNMENT INTEREST:
(0001) This invention was made with government support by the following National Institute of Health Grant DK38961. The government may own certain rights in the present invention

NUMBER OF CLAIMS: 41 1 Figure(s).
DESCRIPTION OF FIGURES:
FIG. 1 shows calbindin-D28k protection against dexamethasone induced cell death of MLOY4 osteocytic cells.

AB The present invention provides novel compositions containing a calbindin-D28k therapeutic element, which is involved in the regulation of apoptosis, and may be administered for the prevention of an abnormal apoptosis response in cells. In particular the compositions and methods of the present invention may be used for the prevention or induction of apoptosis in such cells types as osteoblasts and osteocytes. Specifically, the compositions and methods of the present invention are useful for the prevention of diseases associated with glucocorticoid induced cell death. Specifically, the compositions and methods of the present invention may be useful in the prevention of glucocorticoid induced cell death in osteoblasts and the treatment of such conditions as glucocorticoid induced osteoporosis.

CLMN 41 1 Figure(s).
FIG. 1 shows calbindin-D28k protection against dexamethasone induced cell death of MLOY4 osteocytic cells.

L46 ANSWER 25 OF 58 IFIPAT COPYRIGHT 2005 IFI on STN

AN	10431909 IFIPAT;IFIUDB;IFICDB
TITLE:	METHODS AND COMPOSITIONS USEFUL FOR INHIBITION OF ANGIOGENESIS; USING VITRONECTIN ALPHA V BETA 3 ANTAGONISTS, AND PARTICULARLY FOR INHIBITING ANGIOGENESIS IN INFLAMED TISSUES AND IN TUMOR TISSUES AND METASTASES
INVENTOR(S):	Brooks; Peter, Hollywood, CA, US Cheresh; David A., Encinitas, CA, US
PATENT ASSIGNEE(S):	The Scripps Research Institute, La Jolla, CA, 92037, US
AGENT:	THE SCRIPPS RESEARCH INSTITUTE, OFFICE OF PATENT COUNSEL, TPC-8, 10550 NORTH TORREY PINES ROAD, LA JOLLA, CA, 92037, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2003176334	A1	20030918
APPLICATION INFORMATION:	US 2002-115223		20020402

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
Section 371 PCT Filing OF:	WO 1997-US9158	19970530	PENDING
CONTINUATION OF:	US 1999-194468	19990323	6500924
CONTINUATION-IN-PART OF:	US 1994-210715	19940318	5753230
CONTINUATION-IN-PART OF:	US 1994-366665	19941230	5766591
FAMILY INFORMATION:	US 2003176334	20030918	
	US 6500924		
	US 5753230		
	US 5766591		

DOCUMENT TYPE:	Utility Patent Application - First Publication
FILE SEGMENT:	CHEMICAL APPLICATION
NUMBER OF CLAIMS:	43 36 Figure(s).

DESCRIPTION OF FIGURES:

FIGS. 1A-1D illustrate the tissue distribution of the integrin subunits, beta 3 and beta 1, in normal skin and in skin undergoing wound healing designated as granulation tissue. Immunohistochemistry with antibodies to beta 3 and beta 1 was performed as described in Example 3A. FIGS. 1A and 1B respectively illustrate the immunoreactivity of anti-beta 3 in normal skin and granulation tissue. FIGS. 1C and 1D respectively illustrate the immunoreactivity of anti-beta 1 in normal skin and granulation tissue.

FIGS. 2A-2D illustrate the tissue distribution of the von Willebrand factor and laminin ligands that respectively bind the beta 3 and beta 1 integrin subunits in normal skin and in skin undergoing wound healing designated as granulation tissue. Immunohistochemistry with antibodies to von Willebrand factor (anti-vWF) and laminin (anti-laminin) was performed as described in Example 3B. FIGS. 2A and 2B respectively illustrate the immunoreactivity of anti-vWF in normal skin and granulation tissue. FIGS. 2C and 2D respectively illustrate the immunoreactivity of anti-laminin in normal skin and granulation tissue.

FIGS. 3A-3D illustrate the tissue distribution of the vitronectin integrin receptor, alpha v beta 3, in tissue biopsies of bladder cancer, colon cancer, breast cancer and lung cancer, respectively. Immunohistochemistry with the LM609 antibody against alpha v beta 3 was performed as described in Example 3C.

FIG. 4 illustrates a typical photomicrograph of a CAM of this invention devoid of blood vessels in an untreated 10 day old chick embryo. The preparation is described in Example 5B.

FIGS. 5A-5C illustrate the tissue distribution of the integrins beta 1 and alpha v beta 3 in the CAM preparation of this invention. FIG. 5A shows the distribution of the beta 1 subunit in an untreated 10 day old CAM preparation as detected by immunofluorescence immunoreactivity with CSAT, an anti-beta 1 antibody. FIG. 5B shows the distribution of the alpha v beta 3 receptor in an untreated 10 day old CAM preparation as detected by immunofluorescence immunoreactivity with LM609, an antialpha v beta 3 antibody. FIG. 5C shows the distribution of the alpha v beta 3 receptor in an bFGF treated 10 day old CAM preparation as detected by immunofluorescence immunoreactivity with LM609, an anti-alpha v beta 3 antibody. The treatments and results are described in

Example 5C.
FIG. 6 illustrates the quantification in a bar graph of the relative expression of alpha v beta 3 and beta 1 in untreated and bFGF treated 10 day old CAMs as described in Example 6A. The mean fluorescence intensity is plotted on the Y-axis with the integrin profiles plotted on the X-axis.
FIGS. 7A-7C illustrates the appearance of an untreated 10 day old CAM, a bFGF treated CAM, and a TNF alpha treated CAM, respectively, the procedures and results of which are described in Example 6A.
FIGS. 8A-8E illustrate the effect of topical antibody treatment on bFGF-***induced*** angiogenesis in a day 10 CAM as described in Example 7A1). FIG. 8A shows an untreated CAM preparation that is devoid of blood vessels. FIG. 8B shows the infiltration of new vasculature into an area previously devoid of vasculature **induced** by bFGF treatment. FIGS. 8C, 8D and 8E respectively show the effects of antibodies against beta 1 (anti-beta 1; CSAT), alpha v beta 5 (anti-alpha v beta 5; P3G2) and alpha v beta 3 (anti-alpha v beta 3; LM609).
FIGS. 9A-9C illustrate the effect of intravenous injection of synthetic peptide 66203 on angiogenesis **induced** by tumors as described in Example 7E2).
FIG. 9A shows the lack of inhibitory effect of intravenous treatment with a control peptide (control peptide tumor) on angiogenesis resulting from tumor ***induction.*** The inhibition of such angiogenesis by intravenous injection of peptide 66203 (cyclic RGD tumor) is shown in FIG. 9B. The lack of inhibitory effects or cytotoxicity on mature preexisting vessels following intravenous infusion of peptide 66203 in an area adjacent to the tumor-treated area is shown in FIG. 9C (cyclic RGD adjacent CAM).
FIGS. 10A-10C illustrate the effect of intravenous application of monoclonal antibodies to growth factor **induced** angiogenesis as described in Example 7B1). FIG. 10A shows bFGF-**induced** angiogenesis not exposed to antibody treatment (control). No inhibition of angiogenesis resulted when a similar preparation was treated with anti-alpha v beta 5 antibody P3G2 as shown in FIG. 10B. Inhibition of angiogenesis resulted with treatment of anti-alpha v beta 3 antibody LM609 as shown in FIG. 10C.
FIGS. 11A-11C illustrate the effect on embryonic angiogenesis following topical application of anti-integrin antibodies as described in Example 7C. Angiogenesis was not inhibited by treatment of a 6 day CAM with anti-beta 1 and anti-alpha v beta 5 antibodies respectively shown in FIGS. 11A and 11B. In contrast, treatment with the anti-alpha v beta 3 antibody LM609 resulted in the inhibition of blood vessel formation as shown in FIG. 11C.
FIG. 12 illustrates the quantification of the number of vessels entering a tumor in a CAM preparation as described in Example 7D1). The graph shows the number of vessels as plotted on the Yaxis resulting from topical application of either CSAT (antibeta 1), LM609 (anti-alpha v beta 3) or P3G2 (anti-alpha v beta 5).
FIGS. 13A-13D illustrate a comparison between wet tumor weights 7 days following treatment and initial tumor weights as described in Example 9A1)a. Each bar represents the mean+-S.E. of 5-10 tumors per group. Tumors were derived from human melanoma (M21-L) (FIG. 13A), pancreatic carcinoma (Fg) (FIG. 13B), lung carcinoma (UCLAP-3) (FIG. 13C), and laryngeal carcinoma (HEp3) (FIG. 13D) CAM preparations and treated intravenously with PBS, CSAT (anti-beta 1), or LM609 (antialpha v beta 3) The graphs show the tumor weight as plotted on the Y-axis resulting from intravenous application of either CSAT (anti-beta 1), LM609 (anti-alpha v beta 3) or PBS as indicated on the X-axis.
FIGS. 14A and 14B illustrate histological sections of tumors treated with the P3G2 (anti-alpha v beta 5) (FIG. 14A) and LM609 (anti-alpha v beta 3) (FIG. 14B), and stained with hematoxylin and eosin as described in Example 9A1)b. As shown in FIG. 14A, tumors treated with control antibody (P3G2) showed numerous viable and actively dividing tumor cells as indicated by mitotic figures (arrowheads) as well as by multiple blood vessels (arrows) throughout the tumor stroma. In contrast, few if any viable tumor cells or blood vessels were detected in tumors treated with LM609 (anti-alpha v beta 3) in FIG. 14B.
FIGS. 15A-15E correspond to M21L tumors treated with peptides as described in Example 9A2) and are as follows: FIG. 15A, control cyclic RAD peptide (69601); FIG. 15B, cyclic RGD peptide (66203) ; FIG. 15C, adjacent CAM tissue taken from the same embryos treated wish cyclic RGD peptide (66203) and high magnification (13 x) of tumors treated with the control RAD (69601) in FIG. 15D or cyclic RGD peptide (66203) in FIG. 15E. FIG. 15D depicts normal vessels from the RAD control peptide (69601) treated tumor. FIG. 15E depicts examples of disrupted blood vessels from cyclic RGD peptide (66203) treated tumors (arrows).
FIGS. 16A-16E represent inhibition of angiogenesis by antagonists of

angiogenesis in the in vivo rabbit eye model assay as described in Example 10. FIGS. 16A and 16B depict angiogenesis of the rabbit eye in the presence of bFGF and mAb P1F6 (anti-alpha v beta 5). FIG. 16C, 16D and 16E depict inhibition of angiogenesis of the rabbit eye in the presence of bFGF and mAb LM609 (anti-alpha v beta 3).

FIG. 17 represents a flow chart of how the in vivo mouse:human chimeric mouse model was generated as described in Example 11. A portion of skin from a SCID mouse was replaced with human neonatal foreskin and allowed to heal for 4 weeks. After the graft had healed, the human foreskin was inoculated with human tumor cells. During the following 4 week period, a measurable tumor was established which comprised a human tumor with human vasculature growing from the human skin into the human tumor.

FIG. 18 illustrates the percent of single cells derived from mAbtreated and peptide-treated CAMs and stained with Apop Tag as determined by FACS analysis and described in Example 12. The black and stippled bars represent cells from embryos treated 24 hours and 48 hours prior to the assay, respectively. Each bar represents the mean+-S.E. of three replicates. CAMs were treated mAb LM609 (anti-alpha v beta 3), or CSAT (anti-beta 1) , or PBS. CAMs were also treated with cyclic peptide 66203 (cyclo-RGDfV, indicated as Peptide 203) or control cyclic peptide 69601 (cyclo-RADfV, indicated as Peptide 601).

FIGS. 19A and 19B illustrate the combined results of single cell suspensions of CAMs from embryos treated with either CSAT (anti beta 1) (FIG. 19A) or LM609 (ant-alpha v beta 3) (FIG. 19B), stained with Apop Tag and propidium iodide, and analyzed by FACS as described in Example 12C. The Y axis represents Apop Tag staining in numbers of cells (**Apoptosis**), the X axis represents propidium iodide staining (DNA content). The horizontal line represents the negative gate for Apop Tag staining. The left and right panels indicates CAM cells from CSAT (anti-beta 1) (FIG. 19A) and LM609 (anti-alpha v beta 3) (FIG. 19B) treated embryos, respectively. Cell cycle analysis was performed by analysis of approximately 8,000 events per condition.

DESCRIPTION OF FIGURES:

FIGS. 20A-20C represent CAM tissue from CSAT (anti-beta 1) treated embryos and FIGS. 20D-20F represent CAM tissue from LM609 (anti-alpha v beta 3) treated embryos prepared as described in Example 12C. FIGS. 20A and 20D depict tissues stained with Apop Tag and visualized by fluorescence (FITC) superimposed on a D.I.C. image. FIGS. 20B and 20E depict the same tissues stained with mAb LM609 (anti-alpha v beta 3) and visualized by fluorescence (rhodamine). FIGS. 20C and 20F represent merged images of the same tissues stained with both Apop Tag and LM609 where yellow staining represents colocalization. The bar represents 15 and 50 mu m in the left and right panels, respectively.

FIG. 21 shows the result of a inhibition of cell attachment assay with peptide 85189 as described in Example 4A. The effects of the peptide antagonist was assessed over a dosage range of 0.001 to 100 uM as plotted on the X-axis. Cell attachment is plotted on the Y-axis measured at an optical density (O.D.) of 600 nm. Cell attachment was measured on vitronectin-(broken lines) versus laminin-(solid lines) coated surfaces.

FIGS. 22A and 22B show the consecutive cDNA sequence of chicken MMP-2 along with the deduced amino acid sequence shown on the second line. The third and fourth lines respectively show the deduced amino acid sequence of human and mouse MMP-2 as described in Example 4A. The chicken cDNA sequence is listed in SEQ ID NO 29 along with the encoded amino acid sequence that is also presented separately as SEQ ID NO 30. The numbering of the first nucleotide of the 5' untranslated region and the region encoding the proenzyme sequence shown in FIG. 22A as a negative number is actually presented as number 1 in Sequence Listing making the latter appear longer than the figure; however, the nucleotide sequence is the figure is identical in length and sequence to that as presented in the listing with the exception of the numbering. Accordingly, references to nucleotide position for chicken or human MMP-2 in the specification, such as in primers for use in amplifying MMP-2 fragments, are based on the nucleotide position as indicated in the figure and not as listed in the Sequence Listing.

FIG. 23 shows the results in bar-graph form of a solid-phase receptor binding assay of iodinated MMP-2 to bind to alpha v beta 3 with and without the presence of inhibitors as further described in Example 4B. The data is plotted as bound CPM on the Y-axis against the various potential inhibitors and controls.

FIG. 24 shows the specificity of chicken-derived MMP-2 compositions for either the integrin receptors alpha v beta 3 and alpha 11b beta 3 in the presence of MMP-2 inhibitors as further described in Example 4B. The data is presented as

described in the legend in FIG. 23.
FIG. 25 show the effect of chicken MMP-2(410-637) GST fusion protein on bFGF-induced angiogenesis as described in Example 7A3). FIGS. 25A-B and 25C-D respectively shown control (a nonMMP-2 fragment containing fusion protein) and MMP-2 fragment GST fusion protein effects.
FIGS. 26 and 27 both illustrate in bar graph form the angiogenesis index (a measurement of branch points) of the effects of chicken MMP-2(410-637) GST fusion protein (labeled CTMMP-2) versus control (RAP-GST or GST-RAP) on bFGF-created CAMs as described in Example 7A3). Angiogenic index is plotted on the Y-axis against the separate treatments on the X-axis.
FIG. 28 shows the effects of peptides and organic compounds on bFGF-induced angiogenesis as measured by the effect on branch points plotted on the Y-axis against the various treatments on the X-axis, including bFGF alone, and bFGF-treated CAMs with peptides 69601 or 66203 and organic componds 96112, 96113 and 96229, as described in Examples 7B and 14.
FIG. 29 graphically shows the dose response of peptide 85189 on inhibiting bFGF-induced angiogenesis as further described in Example 7B2) where the number of branch points are plotted on the Y-axis against the amount of ***peptide*** **administered** to the embryo on the X-axis.
FIG. 30 shows the inhibitory activity of peptides 66203 (labeled 203) and 85189 (labeled 189) in bFGF-induced angiogenesis in the CAM assay as described in Example 7B2). Controls included no peptide in bFGF-treated CAMS and peptide 69601 (labeled 601). The data is plotted as described in the legend for FIG. 27.
FIGS. 31A-L show the effect of various treatments against untreated CAM preparations over a time course beginning at 24 hours and ending at 72 hours as further described in Example 7B3). Photographs for the categories labeled untreated, bFGF, bFGF+MAID (bFGF treated followed with exposure to chicken MMP2(2-4) GST fusion protein)' and bFGF+control (bFGF treatment followed by chicken MMP-2(10-1) are respectively shown in FIGS. 31A-C, 31D-F, 31G-I, and 31J-L.
FIGS. 32, 33 and 34 respectively show the reduction in tumor weight for UCLAP-3, M21-L and FgM tumors following intravenous exposure to control peptide 69601 and antagonist 85189 as further described in Example 9A. The data is plotted with tumor weight on the Y-axis against the peptide treatments on the Xaxis.
FIG. 35 illustrates the effect of peptides and antibodies on melanoma tumor growth in the chimeric mouse:human model as further described in Example 11B. The peptides assessed included control 69601 (labeled 601) and antagonist 85189 (labeled 189). The antibody tested was LM609. Tumor volume in mm3 is plotted on the Y-axis against the various treatments on the X-axis.
FIGS. 36A and B respectively show the effect of antagonist 85189 (labeled 189) compared to control peptide 69601 (labeled 601) in reducing the volume and wet weight of M21L tumors over a dosage range of 10, 50 and 250 ug/injection as further described in Example 11C.
FIGS. 37A and 37B show the effectiveness of antagonist peptide 85189 (labeled 189 with a solid line and filled circles) against control peptide 69601 (labeled 601 on a dotted line and open squares) at inhibiting M21L tumor volume in the mouse:human model with two different treatment regimens as further described in Example 11C. Tumor volume in mm3 is plotted on the Y-axis against days on the X-axis.
FIGS. 38 through 42 schematically illustrate the various chemical syntheses of organic molecule alpha v beta 3 antagonists as further described in Example 13.
FIGS. 43 and 44 show the effects of various organic molecules on bFGF-induced angiogenesis in a CAM assay as further described in Example 14. Branch points are plotted on the Y-axis against the various compounds used at 250 ug/ml on the X-axis in FIG. 43 and 100 ug/ml in FIG. 44. !

AB The present invention describes methods for inhibition of angiogenesis in tissues using vitronectin alpha v beta 3 antagonists, and particularly for inhibiting angiogenesis in inflamed tissues and in tumor tissues and metastases using therapeutic compositions containing alpha v beta 3 antagonists.

CLMN 43 36 Figure(s).

FIGS. 1A-1D illustrate the tissue distribution of the integrin subunits, beta 3 and beta 1, in normal skin and in skin undergoing wound healing designated as granulation tissue. Immunohistochemistry with antibodies to beta 3 and beta 1 was performed as described in Example 3A. FIGS. 1A and 1B respectively illustrate the immunoreactivity of anti-beta 3 in normal skin and granulation tissue. FIGS. 1C and 1D respectively illustrate the

immunoreactivity of anti-beta 1 in normal skin and granulation tissue.

FIGS. 2A-2D illustrate the tissue distribution of the von Willebrand factor and laminin ligands that respectively bind the beta 3 and beta 1 integrin subunits in normal skin and in skin undergoing wound healing designated as granulation tissue. Immunohistochemistry with antibodies to von Willebrand factor (anti-vWF) and laminin (anti-laminin) was performed as described in Example 3B. FIGS. 2A and 2B respectively illustrate the immunoreactivity of anti-vWF in normal skin and granulation tissue. FIGS. 2C and 2D respectively illustrate the immunoreactivity of anti-laminin in normal skin and granulation tissue.

FIGS. 3A-3D illustrate the tissue distribution of the vitronectin integrin receptor, alpha v beta 3, in tissue biopsies of bladder cancer, colon cancer, breast cancer and lung cancer, respectively. Immunohistochemistry with the LM609 antibody against alpha v beta 3 was performed as described in Example 3C.

FIG. 4 illustrates a typical photomicrograph of a CAM of this invention devoid of blood vessels in an untreated 10 day old chick embryo. The preparation is described in Example 5B.

FIGS. 5A-5C illustrate the tissue distribution of the integrins beta 1 and alpha v beta 3 in the CAM preparation of this invention. FIG. 5A shows the distribution of the beta 1 subunit in an untreated 10 day old CAM preparation as detected by immunofluorescence immunoreactivity with CSAT, an anti-beta 1 antibody. FIG. 5B shows the distribution of the alpha v beta 3 receptor in an untreated 10 day old CAM preparation as detected by immunofluorescence immunoreactivity with LM609, an antialpha v beta 3 antibody. FIG. 5C shows the distribution of the alpha v beta 3 receptor in an bFGF treated 10 day old CAM preparation as detected by immunofluorescence immunoreactivity with LM609, an anti-alpha v beta 3 antibody. The treatments and results are described in Example 5C.

FIG. 6 illustrates the quantification in a bar graph of the relative expression of alpha v beta 3 and beta 1 in untreated and bFGF treated 10 day old CAMs as described in Example 6A. The mean fluorescence intensity is plotted on the Y-axis with the integrin profiles plotted on the X-axis.

FIGS. 7A-7C illustrates the appearance of an untreated 10 day old CAM, a bFGF treated CAM, and a TNF alpha treated CAM, respectively, the procedures and results of which are described in Example 6A.

FIGS. 8A-8E illustrate the effect of topical antibody treatment on bFGF-**induced** angiogenesis in a day 10 CAM as described in Example 7A1). FIG. 8A shows an untreated CAM preparation that is devoid of blood vessels. FIG. 8B shows the infiltration of new vasculature into an area previously devoid of vasculature **induced** by bFGF treatment. FIGS. 8C, 8D and 8E respectively show the effects of antibodies against beta 1 (anti-beta 1; CSAT), alpha v beta 5 (anti-alpha v beta 5; P3G2) and alpha v beta 3 (anti-alpha v beta 3; LM609).

FIGS. 9A-9C illustrate the effect of intravenous injection of synthetic peptide 66203 on angiogenesis **induced** by tumors as described in Example 7E2). FIG. 9A shows the lack of inhibitory effect of intravenous treatment with a control peptide (control peptide tumor) on angiogenesis resulting from tumor **induction**. The inhibition of such angiogenesis by intravenous injection of peptide 66203 (cyclic RGD tumor) is shown in FIG. 9B. The lack of inhibitory effects or cytotoxicity on mature preexisting vessels following intravenous infusion of peptide 66203 in an area adjacent to the tumor-treated area is shown in FIG. 9C (cyclic RGD adjacent CAM).

FIGS. 10A-10C illustrate the effect of intravenous application of monoclonal antibodies to growth factor **induced** angiogenesis as described in Example 7B1). FIG. 10A shows bFGF-**induced** angiogenesis not exposed to antibody treatment (control). No inhibition of angiogenesis resulted when a similar preparation was treated with anti-alpha v beta 5 antibody P3G2 as shown in FIG. 10B. Inhibition of angiogenesis resulted with treatment of anti-alpha v beta 3 antibody LM609 as shown in FIG. 10C.

FIGS. 11A-11C illustrate the effect on embryonic angiogenesis following topical application of anti-integrin antibodies as described in Example 7C. Angiogenesis was not inhibited by treatment of a 6 day CAM with anti-beta 1 and anti-alpha v beta 5 antibodies respectively shown in FIGS. 11A and 11B. In contrast, treatment with the anti-alpha v beta 3 antibody LM609 resulted in the inhibition of blood vessel formation as

shown in FIG. 11C.

FIG. 12 illustrates the quantification of the number of vessels entering a tumor in a CAM preparation as described in Example 7D1). The graph shows the number of vessels as plotted on the Yaxis resulting from topical application of either CSAT (antibeta 1), LM609 (anti-alpha v beta 3) or P3G2 (anti-alpha v beta 5).

FIGS. 13A-13D illustrate a comparison between wet tumor weights 7 days following treatment and initial tumor weights as described in Example 9A1)a. Each bar represents the mean+-S.E. of 5-10 tumors per group. Tumors were derived from human melanoma (M21-L) (FIG. 13A), pancreatic carcinoma (Fg) (FIG. 13B), lung carcinoma (UCLAP-3) (FIG. 13C), and laryngeal carcinoma (HEp3) (FIG. 13D) CAM preparations and treated intravenously with PBS, CSAT (anti-beta 1), or LM609 (antialpha v beta 3) The graphs show the tumor weight as plotted on the Y-axis resulting from intravenous application of either CSAT (anti-beta 1), LM609 (anti-alpha v beta 3) or PBS as indicated on the X-axis.

FIGS. 14A and 14B illustrate histological sections of tumors treated with the P3G2 (anti-alpha v beta 5) (FIG. 14A) and LM609 (anti-alpha v beta 3) (FIG. 14B), and stained with hematoxylin and eosin as described in Example 9A1)b. As shown in FIG. 14A, tumors treated with control antibody (P3G2) showed numerous viable and actively dividing tumor cells as indicated by mitotic figures (arrowheads) as well as by multiple blood vessels (arrows) throughout the tumor stroma. In contrast, few if any viable tumor cells or blood vessels were detected in tumors treated with LM609 (anti-alpha v beta 3) in FIG. 14B.

FIGS. 15A-15E correspond to M21L tumors treated with peptides as described in Example 9A2) and are as follows: FIG. 15A, control cyclic RAD peptide (69601); FIG. 15B, cyclic RGD peptide (66203) ; FIG. 15C, adjacent CAM tissue taken from the same embryos treated wish cyclic RGD peptide (66203) and high magnification (13 x) of tumors treated with the control RAD (69601) in FIG. 15D or cyclic RGD peptide (66203) in FIG. 15E. FIG. 15D depicts normal vessels from the RAD control peptide (69601) treated tumor. FIG. 15E depicts examples of disrupted blood vessels from cyclic RGD peptide (66203) treated tumors (arrows).

FIGS. 16A-16E represent inhibition of angiogenesis by antagonists of angiogenesis in the in vivo rabbit eye model assay as described in Example 10. FIGS. 16A and 16B depict angiogenesis of the rabbit eye in the presence of bFGF and mAb P1F6 (anti-alpha v beta 5). FIG. 16C, 16D and 16E depict inhibition of angiogenesis of the rabbit eye in the presence of bFGF and mAb LM609 (anti-alpha v beta 3).

FIG. 17 represents a flow chart of how the in vivo mouse:human chimeric mouse model was generated as described in Example 11. A portion of skin from a SCID mouse was replaced with human neonatal foreskin and allowed to heal for 4 weeks. After the graft had healed, the human foreskin was inoculated with human tumor cells. During the following 4 week period, a measurable tumor was established which comprised a human tumor with human vasculature growing from the human skin into the human tumor.

FIG. 18 illustrates the percent of single cells derived from mAbtreated and peptide-treated CAMs and stained with Apop Tag as determined by FACS analysis and described in Example 12. The black and stippled bars represent cells from embryos treated 24 hours and 48 hours prior to the assay, respectively. Each bar represents the mean+-S.E. of three replicates. CAMs were treated mAb LM609 (anti-alpha v beta 3), or CSAT (anti-beta 1) , or PBS. CAMs were also treated with cyclic peptide 66203 (cyclo-RGDfV, indicated as Peptide 203) or control cyclic peptide 69601 (cyclo-RADfV, indicated as Peptide 601).

FIGS. 19A and 19B illustrate the combined results of single cell suspensions of CAMs from embryos treated with either CSAT (anti beta 1) (FIG. 19A) or LM609 (ant-alpha v beta 3) (FIG. 19B), stained with Apop Tag and propidium iodide, and analyzed by FACS as described in Example 12C. The Y axis represents Apop Tag staining in numbers of cells (**Apoptosis**), the X axis represents propidium iodide staining (DNA content). The horizontal line represents the negative gate for Apop Tag staining. The left and right panels indicates CAM cells from CSAT (anti-beta 1) (FIG. 19A) and LM609 (anti-alpha v beta 3) (FIG. 19B) treated embryos, respectively. Cell cycle analysis was performed by analysis of approximately 8,000 events per condition.

FIGS. 20A-20C represent CAM tissue from CSAT (anti-beta 1) treated embryos and FIGS. 20D-20F represent CAM tissue from LM609 (anti-alpha v beta 3)

treated embryos prepared as described in Example 12C. FIGS. 20A and 20D depict tissues stained with Apop Tag and visualized by fluorescence (FITC) superimposed on a D.I.C. image. FIGS. 20B and 20E depict the same tissues stained with mAb LM609 (anti-alpha v beta 3) and visualized by fluorescence (rhodamine). FIGS. 20C and 20F represent merged images of the same tissues stained with both Apop Tag and LM609 where yellow staining represents colocalization. The bar represents 15 and 50 mu m in the left and right panels, respectively.

FIG. 21 shows the result of a inhibition of cell attachment assay with peptide 85189 as described in Example 4A. The effects of the peptide antagonist was assessed over a dosage range of 0.001 to 100 uM as plotted on the X-axis. Cell attachment is plotted on the Y-axis measured at an optical density (O.D.) of 600 nm. Cell attachment was measured on vitronectin-(broken lines) versus laminin-(solid lines) coated surfaces.

FIGS. 22A and 22B show the consecutive cDNA sequence of chicken MMP-2 along with the deduced amino acid sequence shown on the second line. The third and fourth lines respectively show the deduced amino acid sequence of human and mouse MMP-2 as described in Example 4A. The chicken cDNA sequence is listed in SEQ ID NO 29 along with the encoded amino acid sequence that is also presented separately as SEQ ID NO 30. The numbering of the first nucleotide of the 5' untranslated region and the region encoding the proenzyme sequence shown in FIG. 22A as a negative number is actually presented as number 1 in Sequence Listing making the latter appear longer than the figure; however, the nucleotide sequence is the figure is identical in length and sequence to that as presented in the listing with the exception of the numbering. Accordingly, references to nucleotide position for chicken or human MMP-2 in the specification, such as in primers for use in amplifying MMP-2 fragments, are based on the nucleotide position as indicated in the figure and not as listed in the Sequence Listing.

FIG. 23 shows the results in bar-graph form of a solid-phase receptor binding assay of iodinated MMP-2 to bind to alpha v beta 3 with and without the presence of inhibitors as further described in Example 4B. The data is plotted as bound CPM on the Y-axis against the various potential inhibitors and controls.

FIG. 24 shows the specificity of chicken-derived MMP-2 compositions for either the integrin receptors alpha v beta 3 and alpha 11b beta 3 in the presence of MMP-2 inhibitors as further described in Example 4B. The data is presented as described in the legend in FIG. 23.

FIG. 25 show the effect of chicken MMP-2(410-637) GST fusion protein on bFGF-induced angiogenesis as described in Example 7A3). FIGS. 25A-B and 25C-D respectively shown control (a nonMMP-2 fragment containing fusion protein) and MMP-2 fragment GST fusion protein effects.

FIGS. 26 and 27 both illustrate in bar graph form the angiogenesis index (a measurement of branch points) of the effects of chicken MMP-2(410-637) GST fusion protein (labeled CTMMP-2) versus control (RAP-GST or GST-RAP) on bFGF-created CAMs as described in Example 7A3). Angiogenic index is plotted on the Y-axis against the separate treatments on the X-axis.

FIG. 28 shows the effects of peptides and organic compounds on bFGF-induced angiogenesis as measured by the effect on branch points plotted on the Y-axis against the various treatments on the X-axis, including bFGF alone, and bFGF-treated CAMs with peptides 69601 or 66203 and organic componds 96112, 96113 and 96229, as described in Examples 7B and 14.

FIG. 29 graphically shows the dose response of peptide 85189 on inhibiting bFGF-induced angiogenesis as further described in Example 7B2) where the number of branch points are plotted on the Y-axis against the amount of **peptide administered** to the embryo on the X-axis.

FIG. 30 shows the inhibitory activity of peptides 66203 (labeled 203) and 85189 (labeled 189) in bFGF-induced angiogenesis in the CAM assay as described in Example 7B2). Controls included no peptide in bFGF-treated CAMS and peptide 69601 (labeled 601). The data is plotted as described in the legend for FIG. 27.

FIGS. 31A-L show the effect of various treatments against untreated CAM preparations over a time course beginning at 24 hours and ending at 72 hours as further described in Example 7B3). Photographs for the categories labeled untreated, bFGF, bFGF+MAID (bFGF treated followed with exposure to chicken MMP2(2-4) GST fusion protein) and bFGF+control (bFGF treatment followed by chicken MMP-2(10-1) are respectively shown in FIGS.

31A-C, 31D-F, 31G-I, and 31J-L.
FIGS. 32, 33 and 34 respectively show the reduction in tumor weight for UCLAP-3, M21-L and FgM tumors following intravenous exposure to control peptide 69601 and antagonist 85189 as further described in Example 9A. The data is plotted with tumor weight on the Y-axis against the peptide treatments on the Xaxis.
FIG. 35 illustrates the effect of peptides and antibodies on melanoma tumor growth in the chimeric mouse:human model as further described in Example 11B. The peptides assessed included control 69601 (labeled 601) and antagonist 85189 (labeled 189). The antibody tested was LM609. Tumor volume in mm3 is plotted on the Y-axis against the various treatments on the X-axis.
FIGS. 36A and B respectively show the effect of antagonist 85189 (labeled 189) compared to control peptide 69601 (labeled 601) in reducing the volume and wet weight of M21L tumors over a dosage range of 10, 50 and 250 ug/injection as further described in Example 11C.
FIGS. 37A and 37B show the effectiveness of antagonist peptide 85189 (labeled 189 with a solid line and filled circles) against control peptide 69601 (labeled 601 on a dotted line and open squares) at inhibiting M21L tumor volume in the mouse:human model with two different treatment regimens as further described in Example 11C. Tumor volume in mm3 is plotted on the Y-axis against days on the X-axis.
FIGS. 38 through 42 schematically illustrate the various chemical syntheses of organic molecule alpha v beta 3 antagonists as further described in Example 13.
FIGS. 43 and 44 show the effects of various organic molecules on bFGF-induced angiogenesis in a CAM assay as further described in Example 14. Branch points are plotted on the Y-axis against the various compounds used at 250 ug/ml on the X-axis in FIG. 43 and 100 ug/ml in FIG. 44. !

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TITLE:	INTRACELLULAR PROTEIN DELIVERY COMPOSITIONS AND METHODS OF USE; COMPLEX OF PROTEIN AND CATIONIC LIPID		
INVENTOR(S):	Felgner; Philip L., Rancho Santa Fe, CA, US Zelphati; Olivier, La Jolla, CA, US		
PATENT ASSIGNEE(S):	Unassigned		
PATENT ASSIGNEE PROBABLE:	Gene Therapy Systems (Probable)		
AGENT:	KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614, US		

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2003054007	A1	20030320
APPLICATION INFORMATION:	US 2000-738046		20001215

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PRIORITY APPLN. INFO.:	US 1999-172441P	19991217 (Provisional)
FAMILY INFORMATION:	US 2003054007	20030320

DOCUMENT TYPE:	Utility Patent Application - First Publication
FILE SEGMENT:	CHEMICAL APPLICATION
NUMBER OF CLAIMS:	23 7 Figure(s).

DESCRIPTION OF FIGURES:
FIG. 1 is a schematic diagram showing the formation of the first ***protein*** **delivery** reagent (Reagent I) from dried cationic lipid film and a monoclonal antibody, binding of the encapsulated antibody to the plasma membrane, and intracellular delivery of the encapsulated antibody.
FIG. 2 is a schematic diagram of the second **protein delivery** reagent (Reagent II). A maleimide-labeled peptide nucleic acid (PNA) clamp is combined with a plasmid to generate a maleimidelabeled plasmid. A reduced antibody is then combined with the maleimide-labeled plasmid which is transfected into cells using conventional DNA transfection reagents.
FIG. 3 is a schematic diagram of pGeneGrip tm vector showing a PNA clamp bound to a PNA binding site on the plasmid.
FIG. 4 is a schematic diagram of a method for producing streptavidin-labeled

plasmid DNA using a biotin-labeled PNA clamp.
FIG. 5 is a schematic diagram of a third **protein delivery** reagent (Reagent III). An activated oligonucleotide is bound to a monoclonal antibody to form an antibody/oligonucleotide conjugate which is then combined with a cationic liposome. The complex is then transfected into cells using conventional DNA transfection reagents.
FIG. 6 is a schematic diagram of a fourth **protein delivery** reagent (Reagent IV). A bifunctional cross-linking reagent such as SPDP is used to conjugate a protein of interest with a maleimide activated cationic lipid. The mixture is then added onto cells leading to cellular uptake of the protein liposome conjugate.
FIG. 7 shows Reagent I mediated delivery of various proteins into Jurkat cells and **induction** of **apoptosis**. The histograms show FACS analysis of cells that were treated with either a BSAphycoerythrin conjugate (BSA-PE), or a mixture of BSA-PE and either granzyme-B, caspase-3, cytochrome-c or caspase-8. The yaxis on these histograms quantifies the amount of the fluorescent BSA-phycoerythrin that enters the cells, and the xaxis quantifies the amount of **apoptosis** using CaspaTag assay.

AB The present invention relates to compositions and methods for intracellular protein delivery. The compositions include a protein operatively associated with a cationic lipid in such a way as to facilitate intracellular delivery of the protein by the cationic lipid, such as by associating directly with a cationic lipid, encapsulating it in a cationic liposome, associating the protein with a lipoplex comprising cationic lipid and nucleic acid, or associating the protein with an anionic polymer that is in association with a cationic lipid. These compositions are useful in delivering antibodies to intracellular proteins to neutralize their activity, and to introduce therapeutically useful proteins, peptides or small molecules.

CLMN 23 7 Figure(s).

FIG. 1 is a schematic diagram showing the formation of the first **protein delivery** reagent (Reagent I) from dried cationic lipid film and a monoclonal antibody, binding of the encapsulated antibody to the plasma membrane, and intracellular delivery of the encapsulated antibody.

FIG. 2 is a schematic diagram of the second **protein delivery** reagent (Reagent II). A maleimide-labeled peptide nucleic acid (PNA) clamp is combined with a plasmid to generate a maleimidelabeled plasmid. A reduced antibody is then combined with the maleimide-labeled plasmid which is transfected into cells using conventional DNA transfection reagents.

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FIG. 7 shows Reagent I mediated delivery of various proteins into Jurkat cells and **induction** of **apoptosis**. The histograms show FACS analysis of cells that were treated with either a BSAphycoerythrin conjugate (BSA-PE), or a mixture of BSA-PE and either granzyme-B, caspase-3, cytochrome-c or caspase-8. The yaxis on these histograms quantifies the amount of the fluorescent BSA-phycoerythrin that enters the cells, and the xaxis quantifies the amount of **apoptosis** using CaspaTag assay.

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TITLE: INTRACELLULAR PROTEIN DELIVERY COMPOSITIONS AND METHODS OF USE; COMPRISES ENCAPSULATION OF PROTEIN WITH CATIONIC LIPOSOME; FOR ANTIBODY DELIVERY

INVENTOR(S): Felgner; Philip L., Rancho Santa Fe, CA, US
Zelphati; Olivier, La Jolla, CA, US
PATENT ASSIGNEE(S): Unassigned
PATENT ASSIGNEE PROBABLE: Gene Therapy Systems (Probable)
AGENT: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614 US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2003008813	A1	20030109
APPLICATION INFORMATION:	US 2002-141535		20020506

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
CONTINUATION-IN-PART OF:	US 2000-738046	20001215	PENDING

	NUMBER	DATE	
PRIORITY APPLN. INFO.:	US 1999-172411P	19991217	(Provisional)
FAMILY INFORMATION:	US 2003008813	20030109	

DOCUMENT TYPE: Utility
Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION
OTHER SOURCE: CA 138:73539

NUMBER OF CLAIMS: 25 9 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 is a schematic diagram showing the formation of the first ***protein*** **delivery** reagent (Reagent I) from dried cationic lipid film and a monoclonal antibody, binding of the encapsulated antibody to the plasma membrane, and intracellular delivery of the encapsulated antibody.

FIG. 2 is a schematic diagram of the second **protein delivery** reagent (Reagent II). A maleimide-labeled peptide nucleic acid (PNA) clamp is combined with a plasmid to generate a maleimidelabeled plasmid. A reduced antibody is then combined with the maleimide-labeled plasmid which is transfected into cells using conventional DNA transfection reagents.

FIG. 3 is a schematic diagram of pGeneGrip tm vector showing a PNA clamp bound to a PNA binding site on the plasmid.

FIG. 4 is a schematic diagram of a method for producing streptavidin-labeled plasmid DNA using a biotin-labeled PNA clamp.

FIG. 5 is a schematic diagram of a third **protein delivery** reagent (Reagent III). An activated oligonucleotide is bound to a monoclonal antibody to form an antibody/oligonucleotide conjugate which is then combined with a cationic liposome. The complex is then transfected into cells using conventional DNA transfection reagents.

FIG. 6 is a schematic diagram of a fourth **protein delivery** reagent (Reagent IV). A bifunctional cross-linking reagent such as SPDP is used to conjugate a protein of interest with a maleimide activated cationic lipid. The mixture is then added onto cells leading to cellular uptake of the protein liposome conjugate.

FIG. 7 shows Reagent I mediated delivery of various proteins into Jurkat cells and **induction** of **apoptosis**. The histograms show FACS analysis of cells that were treated with either a BSAphycoerythrin conjugate (BSA-PE), or a mixture of BSA-PE and either granzyme-B, caspase-3, cytochrome-c or caspase-8. The yaxis on these histograms quantifies the amount of the fluorescent BSA-phycoerythrin that enters the cells, and the xaxis quantifies the amount of **apoptosis** using CaspaTag assay.

FIG. 8 illustrates the uptake of fluorescein labeled IgG by human or mouse dendritic cells when labeled IgG is delivered with Reagent I delivery compositions.

FIG. 9 illustrates the results of an EliSpot assay to determine the effectiveness of **protein delivery** with and without a delivery reagent to promote an immune response.

AB The present invention relates to compositions and methods for intracellular protein delivery. The compositions include a protein operatively associated with a cationic lipid in such a way as to facilitate intracellular delivery of the protein by the cationic lipid,

such as by associating directly with a cationic lipid, encapsulating it in a cationic liposome, associating the protein with a lipoplex comprising cationic lipid and nucleic acid, or associating the protein with an anionic polymer that is in association with a cationic lipid. These compositions are useful in delivering antibodies to intracellular proteins to neutralize their activity, and to introduce therapeutically useful proteins, peptides or small molecules.

CLMN 25 9 Figure(s).

FIG. 1 is a schematic diagram showing the formation of the first **protein delivery** reagent (Reagent I) from dried cationic lipid film and a monoclonal antibody, binding of the encapsulated antibody to the plasma membrane, and intracellular delivery of the encapsulated antibody.

FIG. 2 is a schematic diagram of the second **protein delivery** reagent (Reagent II). A maleimide-labeled peptide nucleic acid (PNA) clamp is combined with a plasmid to generate a maleimidelabeled plasmid. A reduced antibody is then combined with the maleimide-labeled plasmid which is transfected into cells using conventional DNA transfection reagents.

FIG. 3 is a schematic diagram of pGeneGrip tm vector showing a PNA clamp bound to a PNA binding site on the plasmid.

FIG. 4 is a schematic diagram of a method for producing streptavidin-labeled plasmid DNA using a biotin-labeled PNA clamp.

FIG. 5 is a schematic diagram of a third **protein delivery** reagent (Reagent III). An activated oligonucleotide is bound to a monoclonal antibody to form an antibody/oligonucleotide conjugate which is then combined with a cationic liposome. The complex is then transfected into cells using conventional DNA transfection reagents.

FIG. 6 is a schematic diagram of a fourth **protein delivery** reagent (Reagent IV). A bifunctional cross-linking reagent such as SPDP is used to conjugate a protein of interest with a maleimide activated cationic lipid. The mixture is then added onto cells leading to cellular uptake of the protein liposome conjugate.

FIG. 7 shows Reagent I mediated delivery of various proteins into Jurkat cells and **induction** of **apoptosis**. The histograms show FACS analysis of cells that were treated with either a BSAphycoerythrin conjugate (BSA-PE), or a mixture of BSA-PE and either granzyme-B, caspase-3, cytochrome-c or caspase-8. The yaxis on these histograms quantifies the amount of the fluorescent BSA-phycoerythrin that enters the cells, and the xaxis quantifies the amount of **apoptosis** using CaspaTag assay.

FIG. 8 illustrates the uptake of fluorescein labeled IgG by human or mouse dendritic cells when labeled IgG is delivered with Reagent I delivery compositions.

FIG. 9 illustrates the results of an EliSpot assay to determine the effectiveness of **protein delivery** with and without a delivery reagent to promote an immune response.

L46 ANSWER 28 OF 58 USPAT2 on STN

ACCESSION NUMBER: 2003:282651 USPAT2

TITLE: Nucleic acids encoding PRO615

INVENTOR(S): Goddard, Audrey, San Francisco, CA, UNITED STATES
Godowski, Paul J., Burlingame, CA, UNITED STATES
Gurney, Austin L., Belmont, CA, UNITED STATES
Roy, Margaret Ann, San Francisco, CA, UNITED STATES
Wood, William I., Hillsborough, CA, UNITED STATES

PATENT ASSIGNEE(S): Genentech, Inc., South San Francisco, CA, UNITED STATES (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6962797	B2	20051108
APPLICATION INFO.:	US 2001-20445		20011024 (10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2001-918585, filed on 30 Jul 2001, ABANDONED Continuation-in-part of Ser. No. WO 2000-US4341, filed on 18 Feb 2000, PENDING Continuation-in-part of Ser. No. US 380138, ABANDONED A 371 of International Ser. No. WO 1999-US5028, filed on 8 Mar 1999

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-126773P	19990329 (60)
	US 1998-81955P	19980415 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Andres, Janet
ASSISTANT EXAMINER: Seharaseyon, Jegatheesan
LEGAL REPRESENTATIVE: Barnes, Elizabeth M., Kresnak, Mark T., Dreger, Ginger R.
NUMBER OF CLAIMS: 8
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 237 Drawing Figure(s); 237 Drawing Page(s)
LINE COUNT: 20715
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is directed to novel polypeptides and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L46 ANSWER 29 OF 58 USPAT2 on STN
ACCESSION NUMBER: 2003:127092 USPAT2
TITLE: PRO844 polypeptides
INVENTOR(S): Desnoyers, Luc, San Francisco, CA, UNITED STATES
Goddard, Audrey, San Francisco, CA, UNITED STATES
Godowski, Paul J., Hillsborough, CA, UNITED STATES
Gurney, Austin L., Belmont, CA, UNITED STATES
Wood, William I., Hillsborough, CA, UNITED STATES
PATENT ASSIGNEE(S): Genentech, Inc., South San Francisco, CA, UNITED STATES (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6953836	B2	20051011
APPLICATION INFO.:	US 2001-997333		20011115 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2001-941992, filed on 28 Aug 2001, PENDING Continuation of Ser. No. WO 2000-US8439, filed on 30 Mar 2000, PENDING Continuation-in-part of Ser. No. WO 2000-US5004, filed on 24 Feb 2000, PENDING Continuation-in-part of Ser. No. US 380137, ABANDONED A 371 of International Ser. No. WO 1999-US12252, filed on 2 Jun 1999

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-89948P	19980619 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Landsman, Robert
LEGAL REPRESENTATIVE: Kresnak, Mark T., Barnes, Elizabeth M., Dreger, Esq., Ginger R.
NUMBER OF CLAIMS: 11
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 330 Drawing Figure(s); 330 Drawing Page(s)
LINE COUNT: 30859
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is directed to novel polypeptides and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L46 ANSWER 30 OF 58 USPAT2 on STN

ACCESSION NUMBER: 2003:79288 USPAT2

TITLE: Secreted and transmembrane polypeptides and nucleic acids encoding the same

INVENTOR(S): Ashkenazi, Avi J., San Mateo, CA, UNITED STATES
Baker, Kevin P., Darnestown, MD, UNITED STATES
Botstein, David, Belmont, CA, UNITED STATES
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Paoni, Nicholas F., Belmont, CA, UNITED STATES
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Shelton, David L., Oakland, CA, UNITED STATES
Stewart, Timothy A., San Francisco, CA, UNITED STATES
Tumas, Daniel, Orinda, CA, UNITED STATES
Williams, P. Mickey, Half Moon Bay, CA, UNITED STATES
Wood, William I., Hillsborough, CA, UNITED STATES

PATENT ASSIGNEE(S): Genentech, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005124789	A9	20050609
APPLICATION INFO.:	US 2001-978824	A1	20011016 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1998-40220, filed on 17 Mar 1998, GRANTED, Pat. No. US 6391311 Continuation of Ser. No. US 1998-105413, filed on 26 Jun 1998, ABANDONED Continuation of Ser. No. US 1998-168978, filed on 7 Oct 1998, ABANDONED Continuation of Ser. No. US 1998-184216, filed on 2 Nov 1998, ABANDONED Continuation of Ser. No. US 1998-187368, filed on 6 Nov 1998, ABANDONED Continuation of Ser. No. US 1998-202054, filed on 7 Dec 1998, PENDING Continuation of Ser. No. US 1998-218517, filed on 22 Dec 1998, ABANDONED Continuation of Ser. No. US 1999-254465, filed on 5 Mar 1999, GRANTED, Pat. No. US 6410708 Continuation of Ser. No. US 1999-265686, filed on 10 Mar 1999, GRANTED, Pat. No. US 6455283 Continuation of Ser. No. US 1999-267213, filed on 12 Mar 1999, ABANDONED Continuation of Ser. No. US 1999-284291, filed on 12 Apr 1999, ABANDONED Continuation of Ser. No. US 1999-311832, filed on 14 May 1999, ABANDONED Continuation of Ser. No. US 380137, ABANDONED Continuation of Ser. No. US 1999-380138, filed on 25 Aug 1999, ABANDONED Continuation of Ser. No. US 1999-380142, filed on 25 Aug 1999, ABANDONED Continuation of Ser. No. US 2000-709238, filed on 8 Nov 2000, ABANDONED Continuation of Ser. No. US 2000-723749, filed on 27 Nov 2000, GRANTED, Pat. No. US 6620784 Continuation of Ser. No. US 2000-747259, filed on 20 Dec 2000, GRANTED, Pat. No. US 6569645 Continuation of Ser. No. US 2001-816744, filed on 22

Mar 2001, GRANTED, Pat. No. US 6579520 Continuation of Ser. No. US 2001-816920, filed on 22 Mar 2001, ABANDONED Continuation of Ser. No. US 2001-854280, filed on 10 May 2001, PENDING Continuation of Ser. No. US 2001-854208, filed on 10 May 2001, PENDING Continuation of Ser. No. US 2001-872035, filed on 1 Jun 2001, ABANDONED Continuation of Ser. No. US 2001-874503, filed on 5 Jun 2001, ABANDONED Continuation of Ser. No. US 2001-882636, filed on 14 Jun 2001, ABANDONED Continuation of Ser. No. US 2001-886342, filed on 19 Jun 2001, ABANDONED Continuation of Ser. No. US 2001-918585, filed on 30 Jul 2001, ABANDONED

	NUMBER	DATE	
PRIORITY INFORMATION:	WO 1998-US21141	19981007	
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	WO 1999-US106	19990105	
	WO 1999-US5028	19990308	
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	WO 1999-US10733	19990514	
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US 1999-142680P 19990707 (60)
US 1999-145698P 19990726 (60)
US 1999-146222P 19990728 (60)
US 1999-162506P 19991029 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: HELLER EHRMAN WHITE & MCAULIFFE LLP, 275 MIDDLEFIELD ROAD, MENLO PARK, CO, 94025-3506, US
NUMBER OF CLAIMS: 57
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 237 Drawing Page(s)
LINE COUNT: 21332
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is directed to novel polypeptides and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L46 ANSWER 31 OF 58 USPAT2 on STN
ACCESSION NUMBER: 2003:78485 USPAT2
TITLE: Secreted and transmembrane polypeptides and nucleic acids encoding the same
INVENTOR(S): Goddard, Audrey, San Francisco, CA, UNITED STATES
Godowski, Paul J., Burlingame, CA, UNITED STATES
Gurney, Austin L., Belmont, CA, UNITED STATES
Roy, Margaret Ann, San Francisco, CA, UNITED STATES
Wood, William I., Hillsborough, CA, UNITED STATES
PATENT ASSIGNEE(S): Genentech, Inc., South San Francisco, CA, UNITED STATES (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6916648	B2	20050712
APPLICATION INFO.:	US 2001-999833		20011024 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2001-918585, filed on 30 Jul 2001, PENDING Continuation of Ser. No. WO 2000-US4341, filed on 18 Feb 2000, PENDING Continuation-in-part of Ser. No. US 380138, ABANDONED A 371 of International Ser. No. WO 1999-US5028, filed on 25 Aug 1999

	NUMBER	DATE

PRIORITY INFORMATION:	US 1999-131445P	19990428 (60)
	US 1998-82796P	19980423 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	GRANTED	
PRIMARY EXAMINER:	Kemmerer, Elizabeth	
LEGAL REPRESENTATIVE:	Barnes, Elizabeth M., Kresnak, Mark T., Heller Ehrman White & McAuliffe LLP	
NUMBER OF CLAIMS:	14	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	0 Drawing Figure(s); 237 Drawing Page(s)	
LINE COUNT:	20683	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is directed to novel polypeptides and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L46 ANSWER 32 OF 58 DRUGU COPYRIGHT 2005 THE THOMSON CORP on STN

ACCESSION NUMBER: 2003-48818 DRUGU B P

TITLE:	Inhibition of NF-kappaB by a TAT-NEMO-binding domain peptide accelerates constitutive apoptosis and abrogates LPS-delayed neutrophil apoptosis.
AUTHOR:	Choi M; Rolle S; Wellner M; Cardoso M C; Scheidereit C; Luft F C; Kettritz R
CORPORATE SOURCE:	Univ.Berlin-Humboldt
LOCATION:	Berlin, Ger.
SOURCE:	Blood (102, No. 6, 2259-67, 2003) 10 Fig. 41 Ref. CODEN: BLOOAW ISSN: 0006-4971
AVAIL. OF DOC.:	Division of Nephrology, Franz Volhard Clinic, Wiltbergstrasse 50, 13122 Berlin, Germany. (R.K.). (e-mail: kettritz@fvk.charite-buch.de).
LANGUAGE:	English
DOCUMENT TYPE:	Journal
FIELD AVAIL.:	AB; LA; CT
FILE SEGMENT:	Literature

AN 2003-48818 DRUGU B P

AB TAT-I-kappa-B kinase (IKK)-beta/IKK-gamma (NEMO)-binding domain (NBD)peptide inhibited lipopolysaccharide (LPS)-, but not dexamethasone (DEX, both Sigma-Chemical)- or granulocyte-macrophage colony-stimulating factor (GM-COSF, R and D Systems)-, **induced** nuclear factor (NF)-kappa-B activation, and I-kappa-B-alpha up-regulation and degradation in human PMN neutrophils (PMNN) in-vitro. TAT-NBD prevented TNF-alpha (Sigma-Chemical)-, but not IL-8 (R and D Systems)-, stimulated NF-kappa-B activity. TAT-NBD completely abrogated the LPS- and GM-COSF-, but not DEX-, **induced** decrease in PMN **apoptosis**. TAT-NBD dose-dependently decreased constitutive and LPS-**induced** IKK activity. These results provide a proof of principle for **peptide delivery** by TAT-derived protein transduction domains to specifically inhibit NF-kappa-B activity.

ABEX Flourescein isothiocyanate (FITC)-TAT (1 uM) resulted in staining of almost 100% in human PMN. FITC-TAT-NBD and FITC-TAT-Ctrl (mutant form) stained almost 100% of the cells, whereas no staining was observed with free FITC. TAT-NBD and TAT-Ctrl localized throughout the cytoplasm and the nucleus. LPS (100 ng/ml), GM-COSF (20 ng/ml), and DEX (0.1 uM) delayed apoptotic cell death. LPS, GM-COSF, and DEX decreased the number of apoptotic cells from 38% to 19%, 26% and 25%, respectively. Incubation of PMNN with LPS, but not with GM-COSF or DEX, degraded I-kappa-B-alpha. The 1st LPS effect occurred after 30 min and increased up to 120 min. LPS, but not GM-COSF or DEX, **induced** NF-kappa-B activation. An Ab against p50 strongly decreased the upper and lower band with the formation of an intensive supershifted complex.Serum against p65 decreased the upper band with the appearance of a supershifted complex, whereas no change in the intensity of bands was

seen with anti-p52, -c-Rel, -RelB, and -JunB. LPS increased I-kappa-B-alpha by 80-fold, whereas only marginal effects were seen with GM-COSF or DEX. TAT-NBD, but not the control peptide, blocked NF-kappa-B activation and LPS-**induced** I-kappa-B-alpha up-regulation. TAT-NBD (50 and 100 uM) inhibited LPS-**induced** I-kappa-B-alpha degradation. TAT-NBD (100 uM) inhibited TNF-alpha (20 ng/ml)-, but not IL-8-stimulated NF-kappa-b activity. TAT-NBD (1-200 uM) dose-dependently increased the percentage of apoptotic PMNN. TAT-NBD (10 uM) completely abrogated the effect of LPS and GM-COSF, but not of DEX, on PMNN **apoptosis**. TAT-NBD dose-dependently reduced constitutive and LPS-**induced** IKK activity. (LMF/FM)

L46 ANSWER 33 OF 58 TOXCENTER COPYRIGHT 2005 ACS on STN DUPLICATE 5
ACCESSION NUMBER: 2003:151219 TOXCENTER
COPYRIGHT: Copyright 2005 ACS
DOCUMENT NUMBER: CA13922334145K
TITLE: Dieldrin induces apoptosis by promoting caspase-3-dependent proteolytic cleavage of protein kinase Cδ in dopaminergic cells: relevance to oxidative stress and dopaminergic degeneration
AUTHOR(S): Kitazawa, M.; Anantharam, V.; Kanthasamy, A. G.
CORPORATE SOURCE: Department of Biomedical Sciences, Parkinson's Disorder Research Laboratory, Iowa State University, Ames, IA, 50011-1250, USA.
SOURCE: Neuroscience (Oxford, United Kingdom), (2003) Vol. 119, No. 4, pp. 945-964.
CODEN: NRSCDN. ISSN: 0306-4522.
COUNTRY: UNITED STATES
DOCUMENT TYPE: Journal
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 2003:484211
LANGUAGE: English
ENTRY DATE: Entered STN: 20030701
Last Updated on STN: 20050215

AB We previously reported that dieldrin, one of the potential environmental risk factors for development of Parkinson's disease, **induces apoptosis** in dopaminergic cells by generating oxidative stress. Here, the authors demonstrate that the caspase-3-dependent proteolytic activation of protein kinase Cδ (PKCδ) mediates as well as regulates the dieldrin-induced apoptotic cascade in dopaminergic cells. Exposure of PC12 cells to dieldrin (100-300 μM) results in the rapid release of cytochrome C, followed by the activation of caspase-9 and caspase-3 in a time- and dose-dependent manner. The superoxide dismutase mimetic Mn(III)tetrakis(4-benzoic acid)porphyrin chloride significantly attenuates dieldrin-induced cytochrome C release, indicating that reactive oxygen species may contribute to the activation of pro-apoptotic factors. Interestingly, dieldrin proteolytically cleaves native PKCδ into a 41 kDa catalytic subunit and a 38 kDa regulatory subunit to activate the kinase. The dieldrin-induced proteolytic cleavage of PKCδ and induction of kinase activity are completely inhibited by pretreatment with 50-100 μM concns. of the caspase inhibitors benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (Z-VAD-FMK) and benzyloxycarbonyl-Asp-Glu-Val-Asp-fluoromethylketone (Z-DEVD-FMK), indicating that the proteolytic activation of PKCδ is caspase-3-dependent. Addnl., Z-VAD-FMK, Z-DEVD-FMK or the PKCδ specific inhibitor rottlerin almost completely block dieldrin-induced DNA fragmentation. Because dieldrin dramatically increases (40-80-fold) caspase-3 activity, the authors examined whether proteolytically activated PKCδ amplifies caspase-3 via pos. feedback activation. The PKCδ inhibitor rottlerin (3-20 μM) dose-dependently attenuates dieldrin-induced caspase-3 activity, suggesting pos. feedback activation of caspase-3 by PKCδ. Indeed, delivery of catalytically active recombinant PKCδ via a **protein delivery** system significantly activates caspase-3 in PC12 cells. Finally, overexpression of the kinase-inactive PKCδK376R mutant in rat mesencephalic dopaminergic neuronal cells attenuates dieldrin-induced caspase-3 activity and DNA fragmentation, further confirming the pro-apoptotic function of PKCδ in dopaminergic cells. Together, the authors conclude that caspase-3-dependent proteolytic activation of PKCδ is a critical event

in dieldrin-induced apoptotic cell death in dopaminergic cells.

L46 ANSWER 34 OF 58 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 6
ACCESSION NUMBER: 2003:726152 CAPLUS
DOCUMENT NUMBER: 140:196135
TITLE: Induction of insolubility by herpes simplex virus VP22 precludes intercellular trafficking of N-terminal Apoptin-VP22 fusion proteins
AUTHOR(S): Rutjes, Saskia A.; Bosma, Piter J.; Rohn, Jennifer L.; Noteborn, Mathieu H. M.; Wesseling, John G.
CORPORATE SOURCE: AMC Liver Center, Laboratory of Experimental Hepatology, Academic Medical Center, Amsterdam, 1105 BK, Neth.
SOURCE: Journal of Molecular Medicine (Heidelberg, Germany) (2003), 81(9), 558-565
CODEN: JMLME8; ISSN: 0946-2716
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The herpes simplex virus protein VP22 has the intriguing ability to **deliver proteins** from an expressing cell to neighboring cells. Fusion of VP22 to Apoptin, a protein that **induces apoptosis** in tumor cells but not in normal cells, might enhance the delivery of Apoptin. To analyze this hypothesis two fusion proteins of VP22 and full-length Apoptin were constructed, namely VP22-VP3 and VP3-VP22, and their **apoptosis-inducing** ability and intercellular spreading behavior were analyzed by transfection in tumor cells. While both of the Apoptin-VP22 fusion proteins retained the capacity to kill tumor cells, neither of them showed intercellular trafficking. To determine whether the presence of a nuclear localization signal in the C-terminus of Apoptin caused nuclear retention of the fusion protein and the subsequent lack of intercellular spreading, VP22 was fused to the biol. active N-terminal part (residues 1-69) of Apoptin (VP3n), which lacks the nuclear localization signal. However, anal. of the VP3n-VP22 fusion constructs gave no evidence of intercellular transport. A more careful inspection of different fractions of cell lysates expressing Apoptin with or without fusion to VP22 revealed that both the full-length Apoptin protein and its fusion with VP22 are insol. Despite the fact that VP3n was found to be soluble on its own, which could make it amenable to transport by VP22, the VP3n-VP22 fusion proteins were present exclusively in the insol. fraction. We hypothesize that the N-terminal multimerization domain of Apoptin cooperates with VP22 to facilitate aggregation with cellular proteins, thereby inducing insoly. From these results we conclude that, depending on the fusion partner, VP22 can have a neg. effect on the solubility of fusion proteins, which consequently precludes intercellular trafficking. Such properties should be taken into account when establishing new VP22-mediated protein transduction systems.
REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L46 ANSWER 35 OF 58 TOXCENTER COPYRIGHT 2005 ACS on STN DUPLICATE 7
ACCESSION NUMBER: 2004:27342 TOXCENTER
DOCUMENT NUMBER: PubMed ID: 14578948
TITLE: Development of the protein therapeutics using the super anti-cell death factor FNK
AUTHOR(S): Ohta Shigeo
CORPORATE SOURCE: Department of Biochemistry and Cell Biology, Institute of Development and Aging Sciences, Graduate School of Medicine, Nippon Medical School, Japan
SOURCE: Journal of Nippon Medical School = Nihon Ika Daigaku zasshi, (2003 Oct) 70 (5) 442-6.
Journal Code: 100935589. ISSN: 1345-4676.
COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
FILE SEGMENT: MEDLINE
OTHER SOURCE: MEDLINE 2003501546
LANGUAGE: Japanese
ENTRY DATE: Entered STN: 20040210
Last Updated on STN: 20050607

AB A powerful artificial anti-apoptotic factor will be useful for the reproductive therapies for many diseases by prolonging survival of stem cells. For constructing it, we designed the super anti-apoptotic factor by disturbing three intramolecular polar interactions among alpha-helix structures of Bcl-xL. The resultant mutant Bcl-xL, named FNK, was expected to make the pore-forming domain more mobile and flexible than the wild-type. When overexpressed in Jurkat cells, FNK was markedly more potent in prolonging survival following **apoptosis-inducing** treatment with a kind of cell death cytokines (anti-Fas), a protein kinase inhibitor (staurosporine), cell cycle inhibitors (TN-16, camptothecin, hydroxyurea and trichostatin A) or oxidative stress (hydrogen peroxide and paraquat) than wild-type Bcl-xL. Furthermore, the transfectants of FNK became more resistant against a calcium ionophore and even a heat treatment than wild-type Bcl-xL. In addition, FNK showed marked anti-apoptotic activity in CHO and Jurkat cells deprived of serum. Thus, FNK may be the first mutant generated by site-directed mutagenesis of Bcl-xL with an enhance gain-of-function phenotype. Next, we tried to transduce the FNK protein into cells. Protein therapeutics has the advantage of **delivering proteins** in a short period of time. We have engineered the anti-apoptotic bcl-x gene to generate the super anti-apoptotic factor, FNK, with a more powerful cytoprotective activity. In this study, we fused the protein transduction domain (PTD) of the HIV/Tat protein to FNK, and used the construct in an animal model of ischemic brain injury. When added into culture media of human neuroblastoma cells and rat neocortical neurons, PTD-FNK rapidly transduced into cells and localized to mitochondria within 1 hr. It protected the neuroblastomas and neurons against staurosporine-**induced apoptosis** and glutamate-**induced** excitotoxicity, respectively. The cytoprotective activity of PTD-FNK was found at concentrations as low as 0.3 pM. Additionally, PTD-FNK affected the cytosolic movement of calcium ions, which may relate to its neuroprotective action. Immunohistochemical analysis revealed that myc-tagged PTD-FNK (PTD-myc-FNK) injected intraperitoneally into mice can have access into brain neurons. When injected intraperitoneally into gerbils, PTD-FNK prevented delayed neuronal death in the hippocampus caused by transient global ischemia. These results suggest that PTD-FNK has a potential for clinical utility as a novel protein therapeutic strategy to prevent cell death in the brain. Thus, the **protein delivery** system will be useful to make cells survived for a long time during the differentiation of stem cells in the reproductive therapies.

L46 ANSWER 36 OF 58 LIFESCI COPYRIGHT 2005 CSA on STN

ACCESSION NUMBER: 2003:63549 LIFESCI

TITLE: A method for functional evaluation of caspase activation pathways in intact lymphoid cells using electroporation-mediated protein delivery and flow cytometric analysis

AUTHOR: Eksioglu-Demiralp, E.; Kitada, S.; Carson, D.; Garland, J.; Andreef, M.; Reed, J.C.*

CORPORATE SOURCE: The Burnham Institute, 10901 N. Torrey Pines Road, La Jolla, CA 92037, USA; E-mail: jreed@burnham.org

SOURCE: Journal of Immunological Methods [J. Immunol. Methods], (20030401) vol. 275, no. 1-2, pp. 41-56.
ISSN: 0022-1759.

DOCUMENT TYPE: Journal
FILE SEGMENT: F; W3
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The purpose of the study was to develop a rapid technique for determining the functional status of caspase activation pathways in intact lymphocytes. Proteins known to activate caspase-family cell death proteases (cytochrome c; granzyme-B; caspase-8) were introduced into human leukemia and lymphoma cell lines, as well as freshly isolated lymphocytes and leukemia cells, by electroporation. Fluorochrome-labeled proteins with a wide range of molecular weights (from 15 to 150 kDa) were used to evaluate electroporation efficiency by flow cytometry and to compare the efficiency of **protein delivery** using various electroporation conditions. Caspase activity was monitored using a

cleavable, cell-permeable fluorogenic substrate. Conditions were identified for efficient delivery of proteins of +150 kDa into lymphoid cells. Caspase activation **induced** by various proteins was compared in normal and leukemic lymphocytic cells, revealing impaired caspase activation pathways in some malignant cells. We conclude that electroporation of apoptotic proteins into intact lymphoid cells can be used to contrast the status of various caspase activation pathways, thereby providing insights into the pathological defects in **apoptosis** regulation that exist in individual patient specimens.

L46 ANSWER 37 OF 58 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:216148 CAPLUS
DOCUMENT NUMBER: 136:258265
TITLE: A delivery method for the tumor specific apoptosis inducing activity of apoptin
INVENTOR(S): Noteborn, Mathieu Hubertus Maria; Zhang, Ying-Hui; Voorhoeve, Pieter Mathijs; Leliveld, Sirik Rutger
PATENT ASSIGNEE(S): Leadd B.V., Neth.
SOURCE: Eur. Pat. Appl., 18 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1188832	A1	20020320	EP 2000-203115	20000908
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
EP 1186665	A1	20020313	EP 2001-203404	20010910
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
WO 2002020809	A1	20020314	WO 2001-NL664	20010910
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2001094393	A5	20020322	AU 2001-94393	20010910
US 2002061296	A1	20020523	US 2001-949780	20010910

PRIORITY APPLN. INFO.:

EP 2000-203115	A	20000908
EP 2000-203147	A	20000911
US 2000-236117P	P	20000928
WO 2001-NL664	W	20010910

AB The invention relates to the field of apoptosis. A method for inducing apoptosis specifically in transformed or malignant cells by introduction of protein with apoptin like activity into these cells. The invention provides novel therapeutic substances, for example novel therapeutic proteinaceous compds. that can contain apoptin alone or jointly with other proteinaceous protein or protein fragments, especially in those cases when cells are derailed such as cancer-, auto-immune-derived cells.
REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L46 ANSWER 38 OF 58 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:183818 CAPLUS
DOCUMENT NUMBER: 136:227905
TITLE: A delivery method for the tumor specific apoptosis inducing activity of apoptin
INVENTOR(S): Noteborn, Mathieu Hubertus Maria; Voorhoeve, Pieter Mathijs; Zhang, Ying-Hui; Leliveld, Sirik Rutger
PATENT ASSIGNEE(S): Leadd B.V., Neth.
SOURCE: Eur. Pat. Appl., 31 pp.
CODEN: EPXXDW

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1186665	A1	20020313	EP 2001-203404	20010910
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
EP 1188832	A1	20020320	EP 2000-203115	20000908
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

PRIORITY APPLN. INFO.:
EP 2000-203115 A 20000908
EP 2000-203147 A 20000911
US 2000-236117P P 20000928

AB The invention relates to the field of apoptosis. A method for inducing apoptosis specifically in transformed or malignant cells by introduction of protein with apoptin like activity into these cells. The invention provides novel therapeutic substances, for example novel therapeutic proteinaceous compds. that can contain apoptin alone or jointly with other proteinaceous protein or protein fragments, especially in those cases when cells are derailed such as cancer-, auto-immune-derived cells.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L46 ANSWER 39 OF 58 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2002-443695 [47] WPIDS
CROSS REFERENCE: 1997-512306 [47]; 2001-380482 [40]; 2005-064094 [07]
DOC. NO. NON-CPI: N2002-349605
DOC. NO. CPI: C2002-126213
TITLE: Evaluating risk of an individual to develop Alzheimer's disease using cultured neural crest-derived melanocytes and methods of therapy for Alzheimer's disease using peptides that bind to the neurotrophin receptor.
DERWENT CLASS: B04 D16 S03 S05
INVENTOR(S): GILCHREST, B A; YAAR, M
PATENT ASSIGNEE(S): (UYBO-N) UNIV BOSTON
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 2002051988	A1	20020502	(200247)*		19
US 6696303	B2	20040224	(200415)		

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
US 2002051988	A1	CIP of	US 1996-625765	19960329
		CIP of	WO 1997-US4966	19970328
		Cont of	US 1998-163095	19980929
			US 2001-866898	20010529
US 6696303	B2	CIP of	US 1996-625765	19960329
		CIP of	WO 1997-US4966	19970328
		Cont of	US 1998-163095	19980929
			US 2001-866898	20010529

FILING DETAILS:

PATENT NO	KIND		PATENT NO
US 6696303	B2	Cont of	US 6242416

PRIORITY APPLN. INFO: US 1998-163095 19980929; US
1996-625765 19960329; WO
1997-US4966 19970328; US
2001-866898 20010529

AN 2002-443695 [47] WPIDS
CR 1997-512306 [47]; 2001-380482 [40]; 2005-064094 [07]
AB US2002051988 A UPAB: 20050128
NOVELTY - Inhibiting (M1) beta -amyloid binding to the p75 nerve growth factor receptor (NGFR) of a cell, comprising contacting the cell with a substance having the amino acid sequence lysine-glycine-lysine or lysine-glycine-alanine, is new. The substance binds to the p75 NGFR resulting in the inhibition of beta -amyloid protein or beta -amyloid peptide binding to the p75 NGFR.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) inhibiting (M2) p-amyloid-mediated activation of the p75 NGFR by the same procedure as (M1);

(2) decreasing (M3) the risk of beta -amyloid protein-mediated apoptosis of neuronal cells by inhibiting the binding of beta -amyloid protein or beta -amyloid peptide to the p75 NGFR expressed on central nervous system neuronal cells, comprising administering to the vertebrate a peptide having the amino acid sequence lysine-glycine-alanine where the peptide binds to the p75 NGFR and inhibits the binding of beta -amyloid protein or peptide to the p75 NGFR;

(3) identifying (M4) a substance that inhibits beta -amyloid -mediated apoptosis of neural crest-derived cells;

(4) peptide comprising the amino acid sequence lysine-glycine-alanine that inhibits beta -amyloid mediated apoptosis as determined by method (M4);

(5) identifying (M5) a substance that inhibits beta -amyloid protein or peptide binding to the p75 NGFR expressed by neural crest-derived cells, comprising:

(a) culturing melanocytes that express the p75 NGFR;

(b) as in method (M4);

(c) maintaining the test culture of (b) under conditions sufficient for the beta -amyloid protein or peptide or test-substance to bind to the p75 NGFR expressed on the melanocytes;

(d) determining beta -amyloid activation of the p75 NGFR; and

(e) comparing beta -amyloid activation of the p75 NGFR of melanocytes cultured in (b) with beta -amyloid activation of the p75 NGFR of melanocytes in a control culture comprising cells cultured in the absence of test-substance, where a decrease of beta -amyloid activation compared to the control indicates a substance which inhibits beta -amyloid-mediated apoptosis;

(6) peptide comprising the amino acid sequence lysine-glycine-lysine or lysine-glycine-alanine that inhibits binding of beta -amyloid protein or peptide to the p75 NGFR of melanocytes as determined by method (M5);

(7) evaluating (M6) the risk of an individual to develop Alzheimer's disease associated with beta -amyloid protein or peptide activation of the p75 NGFR;

(8) evaluating (M7) the risk of an individual to develop Alzheimer's disease associated with beta -amyloid protein or peptide activation of P75 NGFR, comprising:

(a) culturing epidermal melanocytes expressing the p75 NGFR obtained from the individual, then producing a test culture and culturing a control cell line of epidermal melanocytes expressing the p75 NGFR;

(b) determining the amount of beta -amyloid protein, or precursor protein produced by the test culture and control culture, peptide, to bind to and activate p75 NGFR, and the substance to be tested; and

(c) comparing the amount of beta -amyloid precursor protein or beta -amyloid proteins produced where production of the precursor or protein in the test culture is greater, the individual is at risk for Alzheimer's disease;

(9) evaluating (M8) the risk of an individual to develop Alzheimer's disease associated with beta -amyloid protein or peptide activation of P75 NGFR;

(10) treating Alzheimer's disease, comprising inhibiting the binding of beta -amyloid protein or peptide to the p75 NGFR expressed on neuronal cells, by administering a peptide comprising the sequence lysine-glycine-alanine;

(11) treating Alzheimer's disease by inhibiting expression of the p75+NTR on neuronal cells by contacting the cells with an anti-p75+NTR anti-sense nucleic acid which hybridizes to the p75+NTR and inhibits

receptor expression;

(12) evaluating (M11) the risk of an individual to develop Alzheimer's disease associated with beta -amyloid protein or peptide activation of the p75 NGFR comprising:

(a) obtaining a skin specimen containing melanocytes from the individual and processing the specimen to render it suitable for immunohistochemistry;

(b) contacting the specimen with an anti-p75+NTR antibody and incubating the specimen and antibody to bind to the p75+NTR present in the melanocytes in the specimen; and

(c) quantifying the level of p75+NTR in the specimen where an increase in the test as compared to an age-matched control specimen indicates the individual is at risk;

(13) evaluating (M12) the risk of an individual to develop Alzheimer's disease associated with beta -amyloid protein or peptide activation of the p75 NGFR comprising:

(a) obtaining a skin specimen containing melanocytes from the individual and processing the specimen to render it suitable for in situ hybridization;

(b) contacting the specimen with a labelled nucleic acid probe comprising a nucleic acid sequence encoding the p75+NTR under conditions suitable for hybridization of the probe with p75+NTR present in the melanocytes in the specimen; and

(c) quantifying the level of p75+NTR in the specimen where an increase in the test as compared to an age-matched control specimen indicates the individual is at risk; and

(14) evaluating (M13) the risk of an individual to develop Alzheimer's disease associated with beta -amyloid protein or peptide activation of the p75 NGFR comprising:

(a) obtaining a skin specimen containing melanocytes from the individual and processing the specimen to render it suitable for in situ polymerase chain reaction (PCR);

(b) contacting the specimen with nucleic acid primers comprising a nucleic acid sequence encoding the p75+NTR under conditions suitable for the amplification of the p75+NTR present in the melanocytes in the specimen; and

(c) quantifying the level of p75+NTR in the specimen where an increase in the test as compared to an age-matched control specimen indicates the individual is at risk.

ACTIVITY - Anti-apoptotic.

No biological data is given.

MECHANISM OF ACTION - Inhibition of binding of beta -amyloid protein to P75 NGFR.

USE - For evaluating the risk of an individual to develop Alzheimer's disease and methods of therapy for Alzheimer's disease using peptides that bind to the neurotrophin receptor and competitively inhibit the binding of beta-amyloid to the receptor. In vitro methods are also provided for screening substances and identifying those capable of inhibiting, or decreasing cell apoptosis mediated by beta -amyloid.

ADVANTAGE - Previous methods for diagnosis and drug therapies for Alzheimer's disease have been difficult to achieve. The methods provide a possibility of achieving in vitro diagnosis using cell cultures and permit early diagnosis of Alzheimer's so that treatment can be started before the disease progresses.

Dwg.0/3

L46 ANSWER 40 OF 58 USPAT2 on STN

ACCESSION NUMBER: 2002:343889 USPAT2

TITLE: Secreted and transmembrane polypeptides and nucleic acids encoding the same

INVENTOR(S): Botstein, David, Belmont, CA, UNITED STATES
Goddard, Audrey, San Francisco, CA, UNITED STATES
Godowski, Paul J., Hillsborough, CA, UNITED STATES
Gurney, Austin L., Belmont, CA, UNITED STATES
Roy, Margaret Ann, San Francisco, CA, UNITED STATES
Wood, William I., Hillsborough, CA, UNITED STATES

PATENT ASSIGNEE(S): Genetech, Inc., South San Francisco, CA, UNITED STATES (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6913919	B2	20050705
APPLICATION INFO.:	US 2001-991181		20011116 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2001-941992, filed on 28 Aug 2001, PENDING Continuation of Ser. No. WO 2000-US8439, filed on 30 Mar 2000, PENDING Continuation-in-part of Ser. No. US 380137, PENDING A 371 of International Ser. No. WO 1999-US12252, filed on 2 Jun 1999

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-141037P	19990623 (60)
	US 1998-89600P	19980617 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Kemmerer, Elizabeth
LEGAL REPRESENTATIVE: Kresnak, Mark T., Barnes, Elizabeth M., Dreger, Esq., Ginger R.
NUMBER OF CLAIMS: 15
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 330 Drawing Figure(s); 330 Drawing Page(s)
LINE COUNT: 31042
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is directed to novel polypeptides and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention. ,

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L46 ANSWER 41 OF 58 USPAT2 on STN
ACCESSION NUMBER: 2002:337935 USPAT2
TITLE: PRO1184 polypeptides
INVENTOR(S): Desnoyers, Luc, San Francisco, CA, UNITED STATES
Goddard, Audrey, San Francisco, CA, UNITED STATES
Godowski, Paul J., Hillsborough, CA, UNITED STATES
Gurney, Austin L., Belmont, CA, UNITED STATES
Wood, William I., Hillsborough, CA, UNITED STATES
PATENT ASSIGNEE(S): Genentech, Inc., South San Francisco, CA, UNITED STATES (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6930170	B2	20050816
APPLICATION INFO.:	US 2001-990444		20011114 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2001-941992, filed on 28 Aug 2001, PENDING Continuation of Ser. No. WO 2000-US8439, filed on 30 Mar 2000, PENDING Continuation-in-part of Ser. No. WO 2000-US5841, filed on 2 Mar 2000, PENDING Continuation-in-part of Ser. No. US 380137, PENDING A 371 of International Ser. No. WO 1999-US12252, filed on 2 Jun 1999

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-88741P	19980610 (60)
	US 1999-141037P	19990623 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Spector, Lorraine
ASSISTANT EXAMINER: Jiang, Dong
LEGAL REPRESENTATIVE: Kresnak, Mark T., Barnes, Elizabeth M., Heller Ehrman, LLP
NUMBER OF CLAIMS: 11

EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 330 Drawing Figure(s); 330 Drawing Page(s)
LINE COUNT: 30882
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to novel polypeptides and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L46 ANSWER 42 OF 58 USPAT2 on STN
ACCESSION NUMBER: 2002:287521 USPAT2
TITLE: PRO1184 antibodies
INVENTOR(S): Desnoyers, Luc, San Francisco, CA, UNITED STATES
Goddard, Audrey, San Francisco, CA, UNITED STATES
Godowski, Paul J., Hillsborough, CA, UNITED STATES
Gurney, Austin L., Belmont, CA, UNITED STATES
Wood, William I., Hillsborough, CA, UNITED STATES
PATENT ASSIGNEE(S): Genentech, Inc., South San Francisco, CA, UNITED STATES (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6956108	B2	20051018
APPLICATION INFO.:	US 2001-992598		20011114 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2001-941992, filed on 28 Aug 2001, PENDING Continuation of Ser. No. WO 2000-US8439, filed on 30 Mar 2000, PENDING Continuation-in-part of Ser. No. WO 2000-US5841, filed on 2 Mar 2000, PENDING Continuation-in-part of Ser. No. US 380137, ABANDONED A 371 of International Ser. No. WO 1999-US12252, filed on 25 Aug 1999

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-141037P	19990623 (60)
	US 1998-88741P	19980610 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Kemmerer, Elizabeth
ASSISTANT EXAMINER: Jiang, Doug
LEGAL REPRESENTATIVE: Kresnak, Mark T., Barnes, Elizabeth M., Dreger, Esq., Ginger R.
NUMBER OF CLAIMS: 4
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 330 Drawing Figure(s); 330 Drawing Page(s)
LINE COUNT: 30781
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to novel polypeptides and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L46 ANSWER 43 OF 58 TOXCENTER COPYRIGHT 2005 ACS on STN DUPLICATE 8
ACCESSION NUMBER: 2003:10917 TOXCENTER
COPYRIGHT: Copyright 2005 ACS
DOCUMENT NUMBER: CA13901000816J
TITLE: Protection against ischemic brain injury by protein therapeutics
AUTHOR(S): Asoh, Sadamitsu; Ohsawa, Ikuroh; Mori, Takashi; Katsura,

Ken-Ichiro; Hiraide, Tomoharu; Katayama, Yasuo; Kimura, Megumi; Ozaki, Daiya; Yamagata, Kumi; Ohta, Shigeo
CORPORATE SOURCE: Department of Biochemistry and Cell Biology, Institute of Gerontology, Nippon Medical School, Kawasaki-city, 211-8533, Japan.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (2002) Vol. 99, No. 26, pp. 17107-17112.
CODEN: PNASA6. ISSN: 0027-8424.
COUNTRY: JAPAN
DOCUMENT TYPE: Journal
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 2003:18981
LANGUAGE: English
ENTRY DATE: Entered STN: 20030113
Last Updated on STN: 20050215

AB Preventing massive cell death is an important therapeutic strategy for various injuries and disorders. Protein therapeutics have the advantage of **delivering proteins** in a short period. We have engineered the antiapoptotic bcl-x gene to generate the super antiapoptotic factor, FNK, with a more powerful cytoprotective activity. In this study, we fused the protein transduction domain (PTD) of the HIV/Tat protein to FNK and used the construct in an animal model of ischemic brain injury. When added into culture media of human neuroblastoma cells and rat neocortical neurons, PTD-FNK rapidly transduced into cells and localized to mitochondria within 1 h. It protected the neuroblastomas and neurons against staurosporine-**induced apoptosis** and glutamate-**induced** excitotoxicity, resp. The cytoprotective activity of PTD-FNK was found at concns. as low as 0.3 pM. Addnl., PTD-FNK affected the cytosolic movement of calcium ions, which may relate to its neuroprotective action. Immunohistochem. anal. revealed that myc-tagged PTD-FNK (PTD-myc-FNK) injected i.p. into mice can have access into brain neurons. When injected i.p. into gerbils, PTD-FNK prevented delayed neuronal death in the hippocampus caused by transient global ischemia. These results suggest that PTD-FNK has a potential for clin. utility as a protein therapeutic strategy to prevent cell death in the brain.

L46 ANSWER 44 OF 58 TOXCENTER COPYRIGHT 2005 ACS on STN DUPLICATE 9
ACCESSION NUMBER: 2003:165722 TOXCENTER
COPYRIGHT: Copyright 2005 ACS
DOCUMENT NUMBER: CA13903030313K
TITLE: Development of p53 protein transduction therapy using membrane-permeable peptides and the application to oral cancer cells
AUTHOR(S): Takenobu, Toshihiko; Tomizawa, Kazuhito; Matsushita, Masayuki; Li, Sheng-Tian; Moriwaki, Akiyoshi; Lu, Yun-Fei; Matsui, Hideki
CORPORATE SOURCE: Department of Physiology, Graduate School of Medicine and Dentistry, Okayama University, Okayama, 700-8558, Japan.
SOURCE: Molecular Cancer Therapeutics, (2002) Vol. 1, No. 12, pp. 1043-1049.
CODEN: MCTOCF. ISSN: 1535-7163.
COUNTRY: JAPAN
DOCUMENT TYPE: Journal
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 2003:61245
LANGUAGE: English
ENTRY DATE: Entered STN: 20030708
Last Updated on STN: 20030715

AB Recent studies suggest that several proteins can transverse biol. membranes through protein transduction. The protein transduction domains of these proteins, 10 - 16 residues long, have been identified as critical domains for the protein transduction. Poly-arginine peptide also has the ability of protein transduction. Here, we show that the **protein delivery** system using 11 poly-arginine peptides (11R) is a powerful tool for the transduction of the biol. active tumor suppressor protein, p53, to suppress the proliferation of oral cancer cells. The 11R-fused p53 proteins (11R-p53) effectively penetrated across the plasma

membrane of the cancer cells and translocated into the nucleus. The proteins induced the activity of the p21/WAF promoter and inhibited the proliferation of human oral cancer cells, in which the p53 gene was mutated. The effect was equivalent to that of the adenovirus-mediated p53 gene transduction system. Moreover, 11R-p53 enhanced the cisplatin-dependent **induction** of **apoptosis** of the cells. These data suggest that this protein transduction method may become a promising cancer therapy.

L46 ANSWER 45 OF 58 DRUGU COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2004-37791 DRUGU M
TITLE: Design and tests of an HIV vaccine.
AUTHOR: McMichael A; Mwau M; Hanke T
LOCATION: Oxford, U.K.
SOURCE: Br.Med.Bull. (62, 87-98, 2002) 2 Fig. 41 Ref.
CODEN: BMBUAQ ISSN: 0007-1420
AVAIL. OF DOC.: MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DS, England.
LANGUAGE: English
DOCUMENT TYPE: Journal
FIELD AVAIL.: AB; LA; CT
FILE SEGMENT: Literature
AN 2004-37791 DRUGU M
AB The design and tests of an HIV vaccine are reviewed. T-cell immune responses to HIV are discussed. The effects of cytotoxic T lymphocyte (CTL) vaccines are considered. The mechanisms of generating CD8+ T-cell responses with a vaccine and broadening T-cell response are described. The protective effects of HIV vaccines are explained. The design of an HIV vaccine is a formidable challenge. The main issues are the level of T-cell response obtained, safety, ensuring that the response is broad enough to cope with the virus variability, and keeping the T-cells as active as possible.
ABEX Any vaccine that does not reproduce the trimeric structure is likely to raise Ab to parts of the protein that are not normally exposed. When virus replication is active, there is often a non-specific activation of both CD4+ and CD8+ T-cells with loss of these cells by fas-**induced apoptosis**. If these T-cells are removed by anti-CD8 Ab infusion, in macaques infected with SIV, the virus titer rises sharply, only to fall when the effect of the Ab in removing CD8+ T-cells wears off. CTL cannot prevent virus entering cells, except by secreting the cytokines that bind to CCR5, but this is thought not to be of great impact. A vaccine-**induced** CTL population will allow cells to be infected and then kill them, possibly eliminating the infection. CTL vaccine reduces the challenge dose of the virus to one that the host can control by making an amplified immune response. Live virus vaccines elicit immune responses that are similar to natural virus infection, both humoral, neutralizing Ab, and CD4+ and CD8+ T-cells. Killed virus vaccines and viral subunit protein vaccines are poor at stimulating CD8+ T-cells. Vaccines that **deliver protein** to the cytosol stimulate good CD8+ T-cell responses, particularly effective are transfected DNA and recombinant viruses. The DNA immunizations give measurable CD8+ T-cell responses in most volunteers. The responses get better with time and the strongest are 6 mth and 1 yr after the immunizations. Similar results are reported for SIV in macaques where CTL **induced** by vaccines do not prevent infection, but virus loads can be reduced by 1000-fold compared to controls and animals survive with normal CD4 T-cell counts, while the controls die. (MLM/SDB)

L46 ANSWER 46 OF 58 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2002-083087 [11] WPIDS
DOC. NO. CPI: C2002-025222
TITLE: Delivering molecule into cell for inhibiting cell proliferation and killing target cell, comprises contacting cell with Vpr polypeptide, regulatory protein encoded by HIV type 1, alone or conjugated to molecule.
DERWENT CLASS: B04
INVENTOR(S): DE NORONHA, C M C; GREENE, W C; HENKLEIN, P; SCHUBERT, U;

SHERMAN, M P
PATENT ASSIGNEE(S): (GLAD-N) GLADSTONE INST J DAVID; (HENK-I) HENKLEIN P; (SCHU-I) SCHUBERT U; (REGC) UNIV CALIFORNIA
COUNTRY COUNT: 97
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001090159	A2	20011129	(200211)*	EN	72
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW					
AU 2001074954	A	20011203	(200221)		
US 2002022027	A1	20020221	(200221)		
EP 1290017	A2	20030312	(200320)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR					
US 6664040	B2	20031216	(200382)		
JP 2004501111	W	20040115	(200410)		118
AU 2001274954	A8	20050922	(200570)		

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2001090159	A2		WO 2001-US16943	20010523
AU 2001074954	A		AU 2001-74954	20010523
US 2002022027	A1	Provisional	US 2000-206610P	20000523
		Provisional	US 2001-267827P	20010209
			US 2001-839329	20010420
EP 1290017	A2		EP 2001-941616	20010523
			WO 2001-US16943	20010523
US 6664040	B2	Provisional	US 2000-206610P	20000523
		Provisional	US 2001-267827P	20010209
			US 2001-839329	20010420
JP 2004501111	W		JP 2001-586970	20010523
			WO 2001-US16943	20010523
AU 2001274954	A8		AU 2001-274954	20010523

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2001074954	A	Based on	WO 2001090159
EP 1290017	A2	Based on	WO 2001090159
JP 2004501111	W	Based on	WO 2001090159
AU 2001274954	A8	Based on	WO 2001090159

PRIORITY APPLN. INFO: US 2001-839329 20010420; US 2000-206610P 20000523; US 2001-267827P 20010209

AN 2002-083087 [11] WPIDS

AB WO 200190159 A UPAB: 20020215

NOVELTY - Delivering (M1) a molecule into a cell comprises contacting the cell with a conjugate comprising a Vpr polypeptide (regulatory or auxiliary protein encoded by human immunodeficiency virus type 1) conjugated to the molecule.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) a composition (I) comprising a Vpr polypeptide conjugated to a therapeutic molecule;

(2) a method of killing a target cell by administering a Vpr polypeptide conjugated to a toxin; and

(3) a method of increasing sensitivity to radiation therapy comprising administration of a composition containing the Vpr.

ACTIVITY - Antipsoriatic; Cytostatic; Apoptotic

MECHANISM OF ACTION - T lymphocyte inhibitor. Full length Vpr was

chemically synthesized and fluorescently labeled, purified by reverse phase high performance liquid chromatography (HPLC), and added to the extracellular media of various cell cultures. Flow cytometry and epifluorescence microscopy were employed to monitor cellular uptake of sVpr. The distribution of cells within different phases of the cell cycle was studied by To-Pro-3 dye staining of DNA followed by flow cytometric analysis. sVpr rapidly and strongly transduced freshly isolated primary human cells at nanomolar concentrations. Analysis of freshly isolated mixed cell populations revealed that CD4+, CD8+, and CD3- lymphocytes as well as CD14+ monocytes were equivalently transduced by sVpr. Transduced human Jurkat T cells accumulated in the G2 phase of the cell cycle. Additionally, full length sVpr was found to **induce apoptosis** in cultured T-cells. Further, transduced sVpr concentrated in the nucleic of monocyte-derived macrophages and significantly increased the replication of HIV viruses lacking Vpr in these cells.

USE - (M1) is useful for delivering a polypeptide, polynucleotide (DNA or RNA), or a toxin into a cell, preferably a cancer cell, a cell infected with a pathogen such as lentivirus, HIV or retrovirus, bacterium or a parasite. (I) comprising Vpr polypeptide conjugated to a toxin is useful for killing a cancer cell or a cell infected with a pathogen, where the toxin is further conjugated to a regulatory molecule where contact with the target cell exerts an effect on the regulatory molecule that results in activation of the toxin. (M2) is useful for inhibiting cell proliferation and treating a disorder associated with dysregulated cell growth in a subject. Vpr **polypeptide administered** alone is also useful for increasing sensitivity to radiation therapy in a subject undergoing radiation therapy (claimed). Vpr polypeptide conjugated to a regulatory moiety is useful for modulating the expression of a transgene in a cell. Vpr polypeptide can be administered alone to treat hyperproliferative cell disorders such as malignancies, psoriasis, and other disorders associated with dysregulated cell growth, and Vpr polypeptide alone or conjugated to an antigen is useful for increasing an immune response.

ADVANTAGE - Synthetic Vpr enters cell efficiently, in nanomolar quantities and without requiring denaturing.

Dwg.0/29

L46 ANSWER 47 OF 58 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2001-273309 [28] WPIDS
CROSS REFERENCE: 2003-697526 [66]
DOC. NO. CPI: C2001-082814
TITLE: Peptides that facilitate uptake and cytoplasmic and/or nuclear transport of proteins, DNA and viruses, useful, e.g. for facilitating uptake of antigens in immunogenic compositions.
DERWENT CLASS: B04 D16
INVENTOR(S): FRIZZELL, R; GAMBOTTO, A; GLORIOSO, J C; MI, Z; ROBBINS, P D; MAI, J C
PATENT ASSIGNEE(S): (UYPI-N) UNIV PITTSBURGH; (FRIZ-I) FRIZZELL R; (GAMB-I) GAMBOTTO A; (GLOR-I) GLORIOSO J C; (MIZZ-I) MI Z; (ROBB-I) ROBBINS P D; (MAIJ-I) MAI J C; (UYPI-N) UNIV PITTSBURGH COMMONWEALTH SYSTEM HIGH
COUNTRY COUNT: 95
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001015511	A2	20010308	(200128)*	EN	127
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW					
AU 2000074735	A	20010326	(200137)		
EP 1210362	A2	20020605	(200238)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					

US 2003104622 A1 20030605 (200339)
US 2003219826 A1 20031127 (200378)
US 2005074884 A1 20050407 (200525)
US 6881825 B1 20050419 (200527)

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2001015511	A2		WO 2000-US24034	20000831
AU 2000074735	A		AU 2000-74735	20000831
EP 1210362	A2		EP 2000-963300	20000831
			WO 2000-US24034	20000831
US 2003104622	A1	Provisional	US 1999-151980P	19990901
		Provisional	US 2000-188944P	20000313
		CIP of	US 2000-653182	20000831
			US 2002-75869	20020213
US 2003219826	A1	Provisional	US 1999-151980P	19990901
		Provisional	US 2000-188944P	20000313
		CIP of	US 2000-653182	20000831
		CIP of	US 2002-75869	20020213
			US 2003-366493	20030212
US 2005074884	A1	Provisional	US 1999-151980P	19990901
		Provisional	US 2000-188944P	20000313
		CIP of	US 2000-653182	20000831
		Cont of	US 2002-75869	20020213
			US 2004-926893	20040826
US 6881825	B1	Provisional	US 1999-151980P	19990901
		Provisional	US 2000-188944P	20000313
			US 2000-653182	20000831

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000074735	A	Based on	WO 2001015511
EP 1210362	A2	Based on	WO 2001015511

PRIORITY APPLN. INFO: US 2000-188944P 20000313; US 1999-151980P 19990901; US 2000-653182 20000831; US 2002-75869 20020213; US 2003-366493 20030212; US 2004-926893 20040826

AN 2001-273309 [28] WPIDS
CR 2003-697526 [66]
AB WO 200115511 A UPAB: 20050427
NOVELTY - Peptides (I) that facilitate uptake and cytoplasmic and/or nuclear transport of proteins, DNA and viruses, are new.

DETAILED DESCRIPTION - A peptide (I) having an amino acid sequence selected from 70 defined amino acid sequences ((S1)-(S70)) given in the specification. For example:

KRIIQRILSRNS (S1)
KRIHPRLTRSIR (S2)
PPRLRKRRQLNM (S3)
PIRRRKKLRRLK (S4)
RRQRRTSKLMKR (S5)

INDEPENDENT CLAIMS are also included for the following:

(a) a peptide-cargo (e.g. nucleic acids, amino acids, small molecules and viruses) complex (II) comprising the peptide (I) and a cargo;

(b) a method (III) for identifying peptides capable of cellular internalization of cargo linked to it, comprising:

(i) incubating a target test cell with a peptide display library;

(ii) isolating internalized peptides presented by the peptide display library from the target cell and identifying the peptides;

(iii) synthesizing the peptides;

(iv) linking the peptides to cargo to form peptide-cargo complexes;

(v) incubating the peptide-cargo complex with a target cell; and

(vi) determining the ability of the peptide to facilitate the

cellular internalization of the cargo into the target cell;

(c) an expression cassette (IV) comprising a DNA encoding a fusion protein comprising a leader sequence, a protein of interest and an internalizing peptide comprising an amino acid sequence selected from (S1)-(S70);

(d) a transfer vector (V) comprising the expression cassette (IV);

(e) a method (VI) for inducing synovial cell death, comprising administering a peptide-cargo complex to the synovial cell;

(f) a method (VII) for **inducing apoptosis** in a tumor cell comprising administering a peptide-cargo complex to the tumor cell;

(g) a method (VIII) for reducing white blood cells in arthritic joints comprising administering a peptide-cargo complex to the white blood cells;

(h) a method (IX) for internalizing a GST fusion protein into a cell comprising administering to the cell a peptide-cargo complex and a GST fusion protein;

(i) a kit (X) for internalizing a GST-fusion protein into a cell comprising a peptide-cargo complex;

(j) an immunogen (XI) comprising a peptide-cargo complex in which the peptide comprises an amino acid sequence selected from (S1)-(S70); and

(k) a method (XII) for eliciting an immune response in a subject, comprising administering to a target cell of the subject an immunogen comprising a peptide-cargo complex in which the peptide comprises an amino acid sequence selected from (S1)-(S70).

ACTIVITY - None specified for the peptide (I) per se.

MECHANISM OF ACTION - The peptide (I) facilitates uptake and cytoplasmic and/or nuclear transport of cargos such as proteins, DNA and viruses.

GST-eGFP (glutathione-S-transferase tagged green fluorescent protein) having an additional histidine tag was expressed in Escherichia coli and purified.

The purified GST-eGFP (200 micro l of 0.8 mg/ml/total of 0.16 mg in TBS containing 1 mM CaCl2) was incubated together with 50 micro l pep5 (RRQRRTSKLMKR (S5), 2 mg/ml in TBS) in a total volume of 500 micro l by rotating overnight at 4 deg. C. The mixture was then dialyzed against TBS at 4 deg. C for 2 hours with one change of buffer.

Hig-82 cells were grown to 100% confluency in 12 well plates. The cells were washed twice with 1 ml of TBS containing 1 mM CaCl2 and 10 mM MgCl2 and 0.1% BSA (bovine serum albumin). After the final wash, various dilutions of the glutathione-pep5-GST-eGFP complex were added to the cells, as well as a negative control (GST-eGFP alone) and enriched TBS. The cells were incubated with the complex or controls at 37 deg. C for 2 hours. The cells were then washed with enriched TBS three times and examined by fluorescent microscopy.

It was found that the glutathione-pep5:GST-eGFP complex was very effectively internalized by Hig-82 cells as compared to the GST-eGFP alone, indicating that the glutathione linked internalization peptide was useful for facilitating the uptake of GST proteins to target cells (results displayed as photographs in the specification).

USE - The peptides (I) may be used for:

(1) facilitating the uptake of a cargo in a target cell;

(2) inducing apoptosis in cells (e.g. arthritic cells and tumor cells);

(3) expanding a population of stem cells;

(4) expanding a population of differentiated cells;

(5) stimulating the differentiation of a population of stem cells;

(6) facilitating the integration of adeno-associated virus DNA into the genome of a cell;

(7) facilitating the uptake into a cell, secretion from the cell and subsequent re-uptake into a neighboring cell of a protein;

(8) facilitating the growth of defective viruses in culture;

(9) stimulating the immune response in a subject;

(10) facilitating uptake of any GST fusion protein into a cell;

(11) eliciting an immune response in a subject; and

(12) facilitating the delivery of immunogens (e.g. vaccines), whether protein-based, DNA-based, vector-based and/or viral-based.

ADVANTAGE - The peptides (I) facilitate uptake and cytoplasmic and/or nuclear transport of cargo.

L46 ANSWER 48 OF 58 COPYRIGHT 2005 Gale Group on STN

ACCESSION NUMBER: 1998:294715 NLDB
TITLE: EUROPEAN PATENT DISCLOSURES.
SOURCE: BIOWORLD Today, (8 Dec 1998) Vol. 9, No. 235.
PUBLISHER: American Health Consultants, Inc.
DOCUMENT TYPE: Newsletter
LANGUAGE: English
WORD COUNT: 1927

L46 ANSWER 49 OF 58 COPYRIGHT 2005 Gale Group on STN

ACCESSION NUMBER: 1998:136543 NLDB
TITLE: EUROPEAN PATENT DISCLOSURES
SOURCE: BIOWORLD Today, (3 Jun 1998) Vol. 9, No. 105.
PUBLISHER: American Health Consultants Inc.
DOCUMENT TYPE: Newsletter
LANGUAGE: English
WORD COUNT: 1826

L46 ANSWER 50 OF 58 COPYRIGHT 2005 Gale Group on STN

ACCESSION NUMBER: 1998:106451 NLDB
TITLE: COMPETITIVE STRATEGIES: Cancer Deals Advance Treatment and Diagnosis
SOURCE: Genesis Report-Dx, (1 Nov 1997) Vol. 7, No. 3.
ISSN: 1061-2289.
PUBLISHER: Genesis Group Associates, Inc
DOCUMENT TYPE: Newsletter
LANGUAGE: English
WORD COUNT: 2810

L46 ANSWER 51 OF 58 COPYRIGHT 2005 Gale Group on STN

ACCESSION NUMBER: 97:65759 NLDB
TITLE: EUROPEAN PATENT DISCLOSURES
SOURCE: BIOWORLD Today, (24 Feb 1997) Vol. 8, No. 36.
PUBLISHER: American Health Consultants
DOCUMENT TYPE: Newsletter
LANGUAGE: English
WORD COUNT: 1079

L46 ANSWER 52 OF 58 DRUGU COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 1997-36684 DRUGU P
TITLE: Investigations of a cyclic peptide antagonist of the alphavbeta3 integrin: implications for targeted tumour therapy.
AUTHOR: Allman R; Jiang W G; Cowburn P; Mason M
CORPORATE SOURCE: Univ.Wales
LOCATION: Cardiff, U.K.
SOURCE: Br.J.Cancer (76, Suppl. 1, 20, 1997)
CODEN: BJCAAI ISSN: 0007-0920
AVAIL. OF DOC.: Research Department, Section of Clinical Oncology, Velindre Hospital, Cardiff CF4 7XL, Wales.
LANGUAGE: English
DOCUMENT TYPE: Journal
FIELD AVAIL.: AB; LA; CT
FILE SEGMENT: Literature
AN 1997-36684 DRUGU P
AB As up-regulation of the alphavbeta3 integrin (vitronectin receptor) is associated with increasing metastatic potential in malignant melanoma, the Authors investigated the activity of the cyclic pentapeptide cRGDfV (which is a potent inhibitor of alphavbeta3) on a panel of melanoma cells derived from different stages of tumor progression. cRGDfV inhibited cell adhesion, prevented colony formation and **induced apoptosis**. The peptide had no effect on normal skin fibroblasts. (conference abstract).

ABEX All of the cell-lines expressed high levels of alphavbeta3, with 1 line also expressing low levels of alphavbeta5. cRGDfV inhibited alphavbeta3-mediated cell adhesion with a IC50 value of 0.2 uM; prevented colony formation in 3-dimensional collagen gels; and **induced apoptosis** of cells maintained in 3-dimensional collagen gels. Preliminary investigations into the mechanism of the activity of cRGDfV indicates that peptide binding **induced** a rapid (less than 5 min) dismantling of Focal Adhesion Complexes, phosphorylation of paxillin and was associated with a calcium transient. The potency of cRGDfV was further illustrated by the ability of the peptide to **induce apoptosis** even in the presence of high, levels of bcl2 and that exogenous **peptide administered** to cells already attached and growing on type I collagen-coated surfaces resulted in rapid rounding up and detachment of cells. This observation indicates that the drug may not be relying upon contact inhibition for its effects. Administration of cRGDfV to normal skin derived fibroblasts, which, express low levels of alphavbeta3 and high levels of alphavbeta5, caused no detectable change in morphology or viability of the cells. (E54/RSV)

L46 ANSWER 53 OF 58 COPYRIGHT 2005 Gale Group on STN

ACCESSION NUMBER: 97:224284 NLDB
TITLE: Nicotine's Good Side: Treating Brain Diseases-Part 3
SOURCE: Genesis Report-Rx, (1 Apr 1996) Vol. 5, No. 3.
ISSN: 1061-2270.
PUBLISHER: Genesis Group Associates, Inc
DOCUMENT TYPE: Newsletter
LANGUAGE: English
WORD COUNT: 3200

L46 ANSWER 54 OF 58 COPYRIGHT 2005 Gale Group on STN

ACCESSION NUMBER: 96:396226 NLDB
TITLE: EUROPEAN PATENT DISCLOSURES: Published October 9 & 16 (EPO); October 3 & 10 (WO)
SOURCE: BIOWORLD Today, (18 Nov 1996) Vol. 7, No. 225.
PUBLISHER: American Health Consultants
DOCUMENT TYPE: Newsletter
LANGUAGE: English
WORD COUNT: 1695

L46 ANSWER 55 OF 58 TOXCENTER COPYRIGHT 2005 ACS on STN DUPLICATE 10
ACCESSION NUMBER: 1996:111988 TOXCENTER
COPYRIGHT: Copyright (c) 2005 The Thomson Corporation
DOCUMENT NUMBER: PREV199699231931
TITLE: Negative selection by endogenous antigen and superantigen occurs at multiple thymic sites
AUTHOR(S): Douek, Daniel C.; Corley, Kevin T. T.; Zal, Tomasz; Mellor, Andrew; Dyson, P. Julian; Altmann, Daniel M. [Reprint author]
CORPORATE SOURCE: Transplantation Biol. Group, MRC Clin. Sci. Cent., Royal Postgrad. Medical Sch., Hammersmith Hosp., London W12 0NN, UK
SOURCE: International Immunology, (1996) Vol. 8, No. 9, pp. 1413-1420.
ISSN: 0953-8178.
DOCUMENT TYPE: Article
FILE SEGMENT: BIOSIS
OTHER SOURCE: BIOSIS 1996:509575
LANGUAGE: English
ENTRY DATE: Entered STN: 20011116
Last Updated on STN: 20011116

AB The site of negative selection in the thymus has been inferred from a range of different experiments. Analysis of thymic deletion of V-beta-5+, V-beta-11+ or V-beta-17a+ cells in H-2E transgenic mice led to the theory that negative selection occurs predominantly in the medulla (specifically, through presentation by medullary dendritic cells). Other experiments investigated whether transgenic TCR are deleted at the double-positive (DP) or single-positive stage following encounter with peptide ligand: by

flow cytometric analysis deletion is generally found to occur at the DP thymocyte stage and as these cells are found predominantly in the cortex, it has been inferred that this is the key site of negative selection. The visualization of apoptotic thymocytes in situ has recently been reported for specific examples of negative selection. Using a panel of TCR transgenic lines in which negative selection occurs at different stages of thymocyte development, we have used TUNEL staining to analyse the anatomical sites of thymocyte apoptosis. For the first time we have been able to compare directly the sites of deletion induced by the endogenous cognate peptides or by endogenous superantigen. We show that generalization from the medullary deletion of V-beta-5+, V-beta-11+ or V-beta-17a+ cells by the endogenous superantigens Mtv 8 and 9 and from limited examples of cortical deletion by exogenous **peptide administered** to TCR transgenic mice is over-simplified. Apoptotic thymocytes in mice lacking Mtv superantigens are indeed localized in the cortex. However, when deletion is **induced** by cognate self peptide, **apoptosis** can occur in the cortex, the meulla or at the junction between the two.

L46 ANSWER 56 OF 58 TOXCENTER COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:322588 TOXCENTER
DOCUMENT NUMBER: CRISP-2003-AI044941-04
TITLE: MECHANISM OF CYTOTOXIC CELL GRANULE MEDIATED APOPTOSIS
AUTHOR(S): FROELICH C J
CORPORATE SOURCE: C-FROELICH@NORTHWESTERN.EDU, EVANS NORTHWESTERN HLTHCARE, INS, 2650 RIDGE AVE, EVANSTON, IL 60201:ILLINOIS
SUPPORTING ORGANIZATION (SPONSORING AGENCY): U.S. DEPT. OF HEALTH AND HUMAN SERVICES; PUBLIC HEALTH SERVICE; NATIONAL INSTITUTES OF HEALTH, NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
SOURCE: Crisp Data Base National Institutes of Health.
DOCUMENT TYPE: (Research)
FILE SEGMENT: CRISP
LANGUAGE: English
ENTRY DATE: Entered STN: 20041229
Last Updated on STN: 20041229

AB DESCRIPTION (Adapted from the Investigator's abstract): The immune system uses granule-mediated apoptosis to eliminate foreign cells. The lethal hit involves delivery of granule associated serine proteases such as granzyme B (GrB) to the target cell cytosol by perforin, a pore forming protein. Perforin is postulated to deliver granzymes by acting as a conduit. Experimental support for this model, however, remains elusive. They have accumulated data supporting the concept that perforin acts intracellularly to deliver the granzymes. In this model, endocytosis of both granzyme and perforin by the target cell are necessary for susceptibility to cytotoxic cell-mediated apoptosis. Subsequently, GrB is released to the cytosol by PFN where the granzyme specifically activates executioner procaspases-3 and -7 through a novel two-step mechanism. Furthermore, related work indicates a target cell is exposed to multiple granzyme molecules complexed to its cognate granule-associated proteoglycan, serglycin. The binding, internalization, intracellular trafficking and proteolytic specificity of GrB-serglycin may differ greatly from the results obtained with the free, cationic granzyme. Conceptually they suggest that granule mediated apoptosis mimics strategies adopted by viruses to enter nucleated cells where PFN delivers a macromolecular signaling complex-an array of granzymes toxically linked to serglycin. The overall goal of our application is to learn whether PFN undergoes endocytosis to deliver GrB and to determine whether GrB-serglycin complexes display the same caspase-activating potential as cytotoxic cells using newly described procaspase-3 deficient MCF-7 cells and a stable transfectant expressing this member. The Specific Aims are: 1. To show a target cell requires normal endocytic function for susceptibility to PFN/GrB- as well as CTL-mediated apoptosis. 2. To characterize GRB-SG complexes isolated from granules of YT cells. 3. To determine whether isolated PFN or the form secreted by a cytotoxic cell **induces apoptosis** in a target cell containing only vesicle-associated GrB/SG. 4. To study the vesicular trafficking of GRB-SG complexes. 5. To learn whether delivery of GrB/SG mimics the ordered caspase activation induced by a cytotoxic cell secreting only GrB.

The information will provide novel insights to the phenomenon of intracellular **protein delivery** as well as the biologic function of multimeric enzyme complexes in intracellular proteolysis. Notably, the system described here will clarify for the first time how PFN delivers granule proteins and GrB activates the caspase cascade in whole cells to initiate death program.

L46 ANSWER 57 OF 58 TOXCENTER COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:153337 TOXCENTER
DOCUMENT NUMBER: CRISP-2002-AI44941-03
TITLE: MECHANISM OF CYTOTOXIC CELL GRANULE MEDIATED APOPTOSIS
AUTHOR(S): FROELICH C J
CORPORATE SOURCE: C-FROELICH@NORTHWESTERN.EDU, EVANSTON NORTHWESTERN HLTHCARE, EVANSTON, IL 60201:ILLINOIS
SUPPORTING ORGANIZATION (SPONSORING AGENCY): U.S. DEPT. OF HEALTH AND HUMAN SERVICES; PUBLIC HEALTH SERVICE; NATIONAL INSTITUTES OF HEALTH, NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
SOURCE: Crisp Data Base National Institutes of Health.
DOCUMENT TYPE: (Research)
FILE SEGMENT: CRISP
LANGUAGE: English
ENTRY DATE: Entered STN: 20030708
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AB DESCRIPTION (Adapted from the Investigator's abstract): The immune system uses granule-mediated apoptosis to eliminate foreign cells. The lethal hit involves delivery of granule associated serine proteases such as granzyme B (GrB) to the target cell cytosol by perforin, a pore forming protein. Perforin is postulated to deliver granzymes by acting as a conduit. Experimental support for this model, however, remains elusive. They have accumulated data supporting the concept that perforin acts intracellularly to deliver the granzymes. In this model, endocytosis of both granzyme and perforin by the target cell are necessary for susceptibility to cytotoxic cell-mediated apoptosis. Subsequently, GrB is released to the cytosol by PFN where the granzyme specifically activates executioner procaspases-3 and -7 through a novel two-step mechanism. Furthermore, related work indicates a target cell is exposed to multiple granzyme molecules complexed to its cognate granule-associated proteoglycan, serglycin. The binding, internalization, intracellular trafficking and proteolytic specificity of GrB-serglycin may differ greatly from the results obtained with the free, cationic granzyme. Conceptually they suggest that granule mediated apoptosis mimics strategies adopted by viruses to enter nucleated cells where PFN delivers a macromolecular signaling complex-an array of granzymes toxically linked to serglycin. The overall goal of our application is to learn whether PFN undergoes endocytosis to deliver GrB and to determine whether GrB-serglycin complexes display the same caspase-activating potential as cytotoxic cells using newly described procaspase-3 deficient MCF-7 cells and a stable transfectant expressing this member. The Specific Aims are: 1. To show a target cell requires normal endocytic function for susceptibility to PFN/GrB- as well as CTL-mediated apoptosis. 2. To characterize GRB-SG complexes isolated from granules of YT cells. 3. To determine whether isolated PFN or the form secreted by a cytotoxic cell **induces apoptosis** in a target cell containing only vesicle-associated GrB/SG. 4. To study the vesicular trafficking of GRB-SG complexes. 5. To learn whether delivery of GrB/SG mimics the ordered caspase activation induced by a cytotoxic cell secreting only GrB. The information will provide novel insights to the phenomenon of intracellular **protein delivery** as well as the biologic function of multimeric enzyme complexes in intracellular proteolysis. Notably, the system described here will clarify for the first time how PFN delivers granule proteins and GrB activates the caspase cascade in whole cells to initiate death program.

L46 ANSWER 58 OF 58 TOXCENTER COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:557084 TOXCENTER
DOCUMENT NUMBER: CRISP-2000-AI44941-01A2
TITLE: MECHANISM OF CYTOTOXIC CELL GRANULE MEDIATED APOPTOSIS
AUTHOR(S): FROELICH C J

CORPORATE SOURCE: EVANSTON NORTHWESTERN HLTHCARE, 2650 RIDGE AVE, RM 2206 CTR, EVANSTON, IL 60201:ILLINOIS
SUPPORTING ORGANIZATION (SPONSORING AGENCY): U.S. DEPT. OF HEALTH AND HUMAN SERVICES; PUBLIC HEALTH SERVICE; NATIONAL INSTITUTES OF HEALTH, NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
SOURCE: Crisp Data Base National Institutes of Health.
DOCUMENT TYPE: (Research)
FILE SEGMENT: CRISP
LANGUAGE: English
ENTRY DATE: Entered STN: 20021200
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AB DESCRIPTION (Adapted from the Investigator's abstract): The immune system uses granule-mediated apoptosis to eliminate foreign cells. The lethal hit involves delivery of granule associated serine proteases such as granzyme B (GrB) to the target cell cytosol by perforin, a pore forming protein. Perforin is postulated to deliver granzymes by acting as a conduit. Experimental support for this model, however, remains elusive. They have accumulated data supporting the concept that perforin acts intracellularly to deliver the granzymes. In this model, endocytosis of both granzyme and perforin by the target cell are necessary for susceptibility to cytotoxic cell-mediated apoptosis. Subsequently, GrB is released to the cytosol by PFN where the granzyme specifically activates executioner procaspases-3 and -7 through a novel two-step mechanism. Furthermore, related work indicates a target cell is exposed to multiple granzyme molecules complexed to its cognate granule-associated proteoglycan, serglycin. The binding, internalization, intracellular trafficking and proteolytic specificity of GrB-serglycin may differ greatly from the results obtained with the free, cationic granzyme. Conceptually they suggest that granule mediated apoptosis mimics strategies adopted by viruses to enter nucleated cells where PFN delivers a macromolecular signaling complex-an array of granzymes toxically linked to serglycin. The overall goal of our application is to learn whether PFN undergoes endocytosis to deliver GrB and to determine whether GrB-serglycin complexes display the same caspase-activating potential as cytotoxic cells using newly described procaspase-3 deficient MCF-7 cells and a stable transfectant expressing this member. The Specific Aims are: 1. To show a target cell requires normal endocytic function for susceptibility to PFN/GrB- as well as CTL-mediated apoptosis. 2. To characterize GRB-SG complexes isolated from granules of YT cells. 3. To determine whether isolated PFN or the form secreted by a cytotoxic cell **induces apoptosis** in a target cell containing only vesicle-associated GrB/SG. 4. To study the vesicular trafficking of GRB-SG complexes. 5. To learn whether delivery of GrB/SG mimics the ordered caspase activation induced by a cytotoxic cell secreting only GrB. The information will provide novel insights to the phenomenon of intracellular **protein delivery** as well as the biologic function of multimeric enzyme complexes in intracellular proteolysis. Notably, the system described here will clarify for the first time how PFN delivers granule proteins and GrB activates the caspase cascade in whole cells to initiate death program.

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